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Deproteinization with Papain enzyme improves the bonding performance of self-etch adhesives to eroded dentin

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To evaluate the effect of papain enzyme pretreatment on the bonding performance of self-etch adhesives (SEAs) to eroded dentin, assess its proteinizing effect, and examine the ultrastructure of the pretreated eroded dentin surface and resin-dentin interface. Artificially eroded dentin surfaces were created and pretreated with papain enzyme, while untreated eroded dentin surface served as a control. The treated dentin surfaces were bonded with Clearfil SE Bond 2 (SEB) and Clearfil Universal Bond Quick (UBQ). Microtensile bond strength (μTBS) was measured after 24-hour storage and after 10,000 thermocycles, between 5 °C and 55 °C. Additionally, the deproteinizing effect was evaluated by comparing changes in the amide-to-phosphate ratio using Raman microscopy. Dentin morphology and resin-dentin interface were investigated using scanning electron microscopy. Statistical analysis was performed using three-way ANOVA with Tukey's post hoc tests, and t-tests ($p < 0.05$). Pretreatment with papain enzyme slightly increased the initial μTBS of SEB, while significantly increasing the initial μTBS of UBQ ($p < 0.05$). Furthermore, papain enzyme could stabilize the μTBS of both adhesives after thermocycles ($p > 0.05$). It also significantly reduced the amide-to-phosphate ratio ($p < 0.05$) altered the surface morphology and improved the structure of resin-eroded dentin interfaces. Deproteinization with papain enzyme dissolved the organic components on the eroded dentin surface, leading to the improvement of resin infiltration, increased thickness of the hybrid layer, and improved the bond durability of SEAs to eroded dentin. The application of the papain enzyme as a pretreatment has the potential to enhance and maintain the bonding performance of self-etch adhesives to eroded dentin. This leads to improved adhesion and restoration quality on compromised eroded dentin surfaces.

Keywords Erosion, Dentin adhesion, Raman spectroscopy, Microtensile bond strength, Resin-dentin interface, Deproteinizing effect

Dental erosion presents a significant oral health concern with global prevalence of 20–45% in permanent dentition[1,2]. This condition, characterized by irreversible loss of dental tissue due to chemical acids, increases in severity with age and impacts quality of life[3–5].

Advanced erosive tooth wear leads to dentin exposure and subsequent hypersensitivity, necessitating minimally invasive interventions with resin composites[6–10]. However, the reduction in mineral content and exposed collagen fibrils[11–13], create a selective barrier that impedes adhesive, monomer infiltration and

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hydroxyapatite interaction[13–15], underscoring the necessity for effective surface pretreatments to enhance bonding performance[11,16–18].

Recently, some researchers have demonstrated that pretreatment with papain-based solutions offers an alternative approach to reducing the organic-to-inorganic ratio on dentin surface[24,25]. As a plant-derived cysteine protease, papain effectively breaks down partially degraded collagen molecules[26]. Unlike NaOCl and HOCl, which can compromise bond strength, papain-based deproteinization has shown promising results in improving bond strength and sealing of self-etch adhesives to caries-affected dentin[24,25]. However, its effect on eroded dentin bonding remains uninvestigated.

Self-etch adhesives (SEAs) exhibit superior performance in dentin bonding[27]. Their mild acidity, however, results in limited etching and incorporation of smear layer remnants into the adhesive interface, potentially compromising bond performance on organic-rich surfaces such as eroded and caries-affected dentin[28–31]. Although two-step self-etch adhesive (2-SEA) remains the gold standard, recent developments in rapid bond technology have led to a single-bottle universal adhesive incorporating a new amide monomer. This adhesive offers rapid substrate permeation, reduced application time and minimized water absorption[32]. Although previous studies have shown comparable or superior dentin bond strength between the new amide monomer-containing adhesive and 2-SEA[32,33], its effectiveness on organic-rich erosive dentin remains limited.

To date, no studies have examined the effect of papain enzyme on the bond performance of SEAs to eroded dentin. Therefore, this study aimed to evaluate the effect of papain enzyme pretreatment on the bond performance of SEAs to eroded dentin after 24-hour storage and thermocycling. The investigation compared the gold standard, 2-SEA with the single bottle universal adhesive containing new amide monomer. Additionally, deproteinizing effect of papain enzyme was evaluated by comparing changes in the amide-to-phosphate ratio, as determined using Raman microscopy, ultrastructure of pretreated eroded dentin surface and resin-dentin interface were investigated using scanning electron microscopy (SEM). The null hypotheses were that the papain enzyme pretreatment would not: (1) improve bond performance of SEAs to eroded dentin; (2) reduce the amide-to-phosphate ratio; (3) alter the ultrastructure of pretreated eroded dentin surface; and (4) improve the characteristics of resin-dentin interface.

Materials and methods

The materials used in this study included a 2-SEA (Clearfil SE Bond 2; SEB, Kuraray Noritake Dental, Tokyo, Japan), a universal adhesive (Clearfil Universal Bond Quick; UBQ, Kuraray Noritake Dental, Tokyo, Japan), a resin composite (Clearfil AP-X shade A2; Kuraray Noritake Dental, Tokyo, Japan), and a 10 wt% papain enzyme containing gel (BRIX 3000®; Brix Medical Science, Argentina). The overview is presented in Table 1.

The study was conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments. The use of extracted human teeth in this study was approved by the Human Research Ethics Committee of the faculty of Dentistry Chulalongkorn University under protocol number 103/2023 and 022/2024.

Sample Preparation

Sixty one caries-free, extracted human molars stored in distilled water at 4 °C were used for the μTBS test within six months. Dentin surfaces were ground flat using a model trimmer under water cooling, and a standardized smear layer was created by grinding with a 600-grit SiC paper under running water for 30 s. The artificially eroded dentin surfaces were created by an erosive cycling model.

Erosive cycling model

The dentin surfaces were subjected to simulated erosive challenge, following the protocol described by Cardenas et al. in 2021 [34]. Before erosive cycling, the lateral and root surfaces were covered with two layers of nail varnish (Revlon, Inc.) to allow erosion only on the occlusal surface. Each specimen was immersed in 10 mL of soft drink (Original Coca-Cola, pH 2.32) for 90 s four times per day for five days. After each erosive cycle with soft drink,

Material (manufacturer)	Batch number	Composition	Application procedure
Clearfil SE Bond 2 (Kuraray Noritake Dental, Tokyo, Japan)	3C0143 2J0208	Primer: 10-MDP, HEMA, hydrophilic aliphatic dimethacrylate, dl-CQ, water Bond: 10-MDP, Bis-GMA, HEMA, dl-CQ, hydrophobic aliphatic dimethacrylate, initiators, silanated colloidal silica	1. Apply primer and leave for 20 s Air-dry for 10 s 2. Apply bond and make a uniform bond film using a gentle air flow 3. Light-cure for 10 s
Clearfil Universal Bond Quick (Kuraray Noritake Dental, Tokyo, Japan)	610413	10-MDP, Bis-GMA, HEMA, hydrophilic amide monomers, colloidal silica, ethanol, dl-CQ, accelerators, silane coupling agent, water, sodium fluoride	1. Apply adhesive with a rubbing motion (no waiting time) 2. Dry with gentle air for 5 s 3. Light cure for 10 s
BRIX 3000® (Brix Medical Science, Argentina)	100223	10 wt% papain 30,000 U/mg, propylene glycol, citric pectin, triethanolamine, sorbitan monolaurate, monopotasic phosphate, toluidine blue, distilled water	1. Apply for 30 s 2. Wash with water for 30 s 3. Air-dry for 5 s
Clearfil AP-X (Kuraray Noritake Dental, Tokyo, Japan)	CM0115	Bis-GMA, TEGDMA, CQ, photoinitiators, pigments, silanated barium glass, silanated silica	1. Apply resin composite in thickness less than 2 mm 2. Light cure for 20 s 3. Repeat 3 times

Table 1. Materials used in this study. 10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate, HEMA: 2-hydroxyethyl methacrylate, CQ: camphorquinone, Bis-GMA: bisphenol A-glycidyl methacrylate, TEGDMA: triethyleneglycol dimethacrylate.

the specimens were rinsed with deionized water for 10 s and immersed in 10 mL of remineralization solution (4.08 mM H_3PO_4, 20.10 mM KCl, 11.90 mM Na_2CO_3, and 1.98 mM $CaCl_2$, pH 6.7) for 1 h[35]. The specimens were stored in remineralization solution at 37 °C after finishing the cycles each day.

Microtensile bond strength (μTBS) test

Forty artificially eroded dentin surfaces were randomly assigned to four experimental conditions ($n = 10$ teeth) according to pretreatment methods and adhesive techniques to measure the μTBS and durability. The experimental design for μTBS is illustrated in Fig. 1.

The deproteinizing pretreatment was done using papain enzyme (BRIX 3000®; Brix Medical Science, Argentina) applied with a disposable microbrush for 30 s, washed-out with water for 30 s, and air-dried for 5 s, while untreated eroded dentin surface served as a control. The adhesives, including SEB and UBQ were applied according to the manufacturer's instructions (Table 1) and light-cured for 10 s using a LED light-curing unit (1000 mW/cm^2, Valo, Ultradent, South Jordan, UT, USA). The bonded dentin surfaces were built up with three increments of 2-mm resin composite (Clearfil AP-X, shade A2; Kuraray Noritake Dental, Tokyo Japan) that were light-cured for 20 s each.

After 24 h of storage in water at 37 °C, each bonded specimen was sectioned parallel to the long axis of the tooth into beam shapes (bonded surface area of 1.0 ± 0.1 mm^2) using a slow-speed diamond saw with water cooling

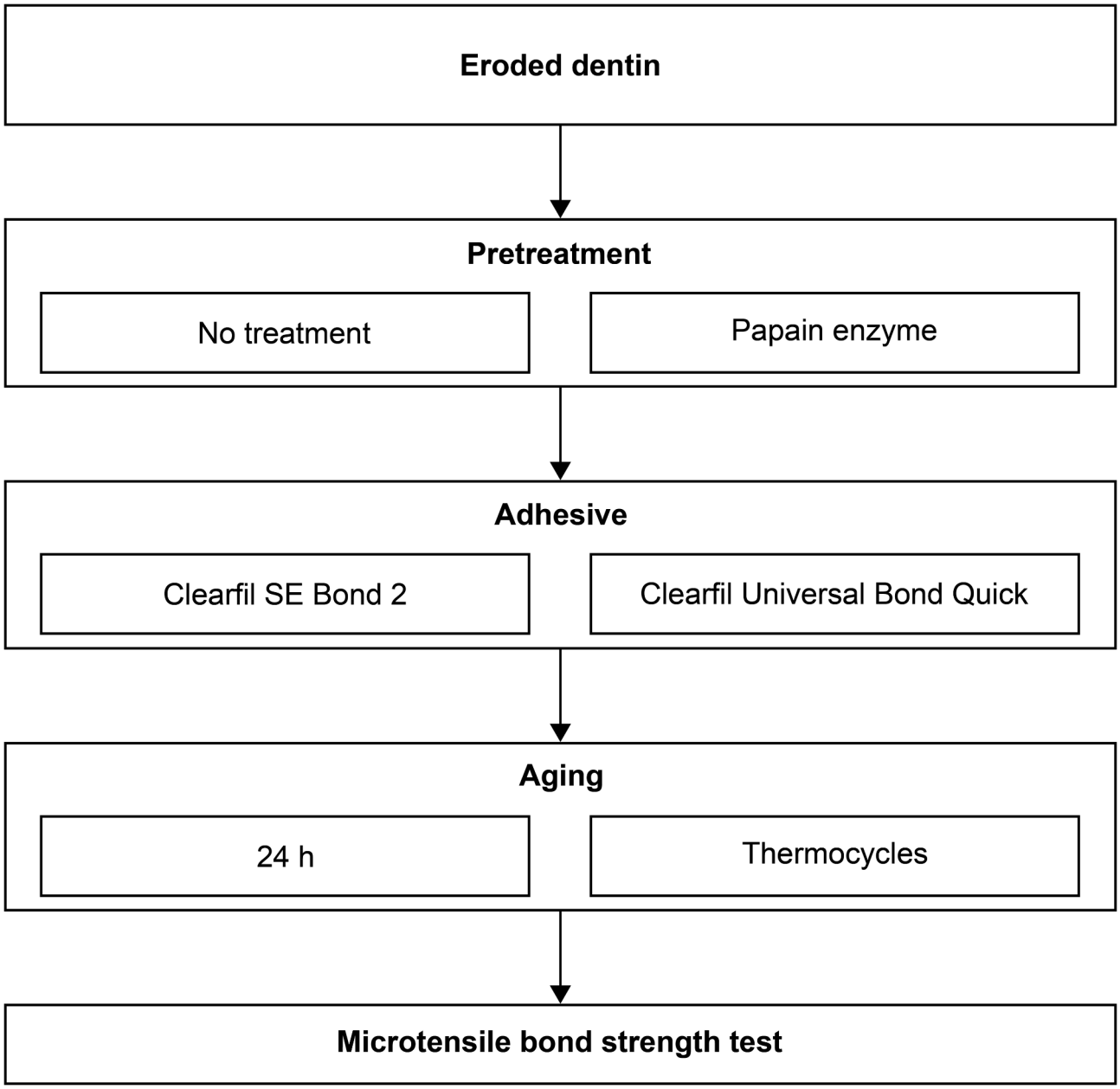

Fig. 1. Design of the microtensile bond strength test.

(Isomet, Buehler, Lake Bluff, IL, USA). Four beams at the center of the specimen were used and randomly divided into two groups: 24-hour water storage and artificial aging by 10,000 thermocycles (TC, Fig. 1). Thermocycling was done in accordance with the Academy of Dental Materials guidance on μTBS testing[36] between 5 °C and 55 °C, with the dwell time of 30 s in each bath and transfer time of 5 s. After the designated aging procedure, beams were attached to a universal testing machine (EZ-S, Shimadzu, Kyoto, Japan) and subjected to the μTBS test at a crosshead speed of 1 mm/minute.

In the statistical analysis, beams were considered statistical units ($n = 20$ beams). The Kolmogorov-Smirnov test and Levene's test indicated that the μTBS data were normal distributed and had homogeneous variance respectively. The data were analyzed using a three-way ANOVA (variables: pretreatment, adhesive, and aging) with Tukey's post hoc test. The bonding durability was analyzed by comparing μTBS after 24-hour storage and TC in each group using t-tests. The analyses were performed at a significance level of 0.05 using SPSS (version 29.0.1 IBM, Chicago, IL, USA).

Fractography analysis

After the μTBS, both the dentin and composite surfaces of all fractured specimens were desiccated in a desiccator for 24 h before evaluation. The dried specimens were sputter-coated with gold and observed using a SEM (Quanta 250, FEI, USA). Failure modes were classified as follows: adhesive failure (> 80% of the fracture occurred between the adhesive and dentin); cohesive failure in dentin (> 80% of the fracture occurred in the dentin); cohesive failure in resin (> 80% of the fracture occurred in the adhesive and/ or the overlying resin composite); or mixed failure (combination of adhesive and cohesive failure). The distribution of failure modes was statistically analyzed using the non-parametric Pearson's chi-square test at a significance level of 0.05.

SEM observation of pretreated eroded dentin surface

Six artificially eroded dentin surfaces, conducted from the erosive cycling model, were used to observe the ultrastructure of pretreated eroded dentin surfaces using SEM. The eroded dentin surfaces were pretreated with papain enzyme as mentioned in "μTBS" section, while non-pretreated served as the control ($n = 3$ teeth). The specimens were fixed using 2.5% glutaraldehyde in phosphate-buffered saline for 2 h at 4 °C, and serially dehydrated in an ascending series of ethanol as follows: 50%, 70% and 80% ethanol for 25 min each at 4 °C, then 90% and 95% ethanol for 25 min each at room temperature, and finally 100% ethanol twice for 25 min. After immersion in hexamethyldisilane (HMDS) for 10 min, the specimens were mounted on metal stubs and dried in a desiccator at room temperature for 24 h. The specimens were sputter-coated with gold and observed using SEM (Quanta 250, FEI, Oregon, USA) at 10,000x magnification.

SEM observation of resin-dentin interface

Additionally, bonded specimens ($n = 3$) were obtained according to the bonding method described in "μTBS" section. Each bonded specimen was sectioned into 1.5-mm-thick slices perpendicular to the bonding interface, with two central slices selected for the SEM observation. The resin-dentin slices were then polished sequentially with 600-, 800-, 1000-, 1500-, and 2000-grit SiC paper respectively, and treated with 37% phosphoric acid for 30 s, followed by immersion in 5.25% sodium hypochlorite for 10 min. After ultrasonic cleaning with deionized water, the resin-dentin slices were dehydrated in a desiccator, sputter-coated with gold and observed using SEM (Quanta 250, FEI, Oregon, USA) at 3,000x magnification.

Investigation of deproteinizing effect using Raman spectroscopy

The roots of three artificially eroded dentin teeth were removed to fabricate 2-mm-thick dentin discs. Raman spectra were collected from the untreated eroded dentin surfaces using a confocal Raman microscope (Renishaw InVia, Renishaw plc, Gloucheshire, UK) with a laser wavelength of 785 nm, an exposure time of 10 s, a magnification of 50x, and spectral ranges from 700 cm^{-1} to 1900 cm^{-1}. Spectra were obtained from four selected sites at the center, with five spectra per site. After pretreatment according to the method mentioned in "μTBS" section, the spectra were collected again under the same conditions ($n = 3$).

The deproteinizing effect was evaluated by calculating the amide-to-phosphate ratio[22], defined as the ratio of C = O stretching vibrations of amide I at 1670 cm^{-1} (representing collagen) to ν_1 P–O stretching vibrations at 960 cm^{-1} (representing apatite)[37]. The decrease in the amide-to-phosphate ratio indicates organic removal from the dentin surfaces[21,22]. The data were statistically analyzed using paired sample t-test at a significance level of 0.05.

Results

μTBS

Mean μTBS values and standard deviations are presented in Table 2. The three-way ANOVA revealed that μTBS were significantly influenced by pretreatment ($p < 0.001$), adhesive ($p < 0.001$), and aging ($p = 0.003$). Interactions between pretreatment and adhesive, as well as between pretreatment and aging factors were significant ($p < 0.001$ and $p = 0.005$, respectively), but interaction between adhesive and aging, and between three factors were not significant ($p = 0.80$ and $p = 0.77$, respectively). SEB exhibited significantly higher μTBS than UBQ in any treatment protocol ($p < 0.05$). Papain enzyme pretreatment led to a slight increase in initial μTBS of SEB ($p = 0.08$) and maintained these values after TC ($p = 0.94$), while specimens without pretreatment showed significant bond strength reduction after TC ($p = 0.003$). For UBQ, papain enzyme pretreatment significantly increased the initial μTBS of UBQ ($p < 0.001$) and maintained bond stability after TC ($p = 0.92$), while specimens without pretreatment exhibited significant bond strength reduction after TC ($p = 0.03$).

Adhesive	Pretreatment	24 h	TC
SEB	No	63.5 ± 7.1^{ACa}	55.5 ± 8.5^{Ab}
	Papain	70.4 ± 7.7^{Aa}	70.2 ± 5.4^{Ba}
UBQ	No	30.1 ± 11.1^{Ba}	23.5 ± 7.3^{Cb}
	Papain	56.8 ± 9.3^{Ca}	56.5 ± 4.6^{Aa}

Table 2. Means and standard deviations of microtensile bond strength (MPa).

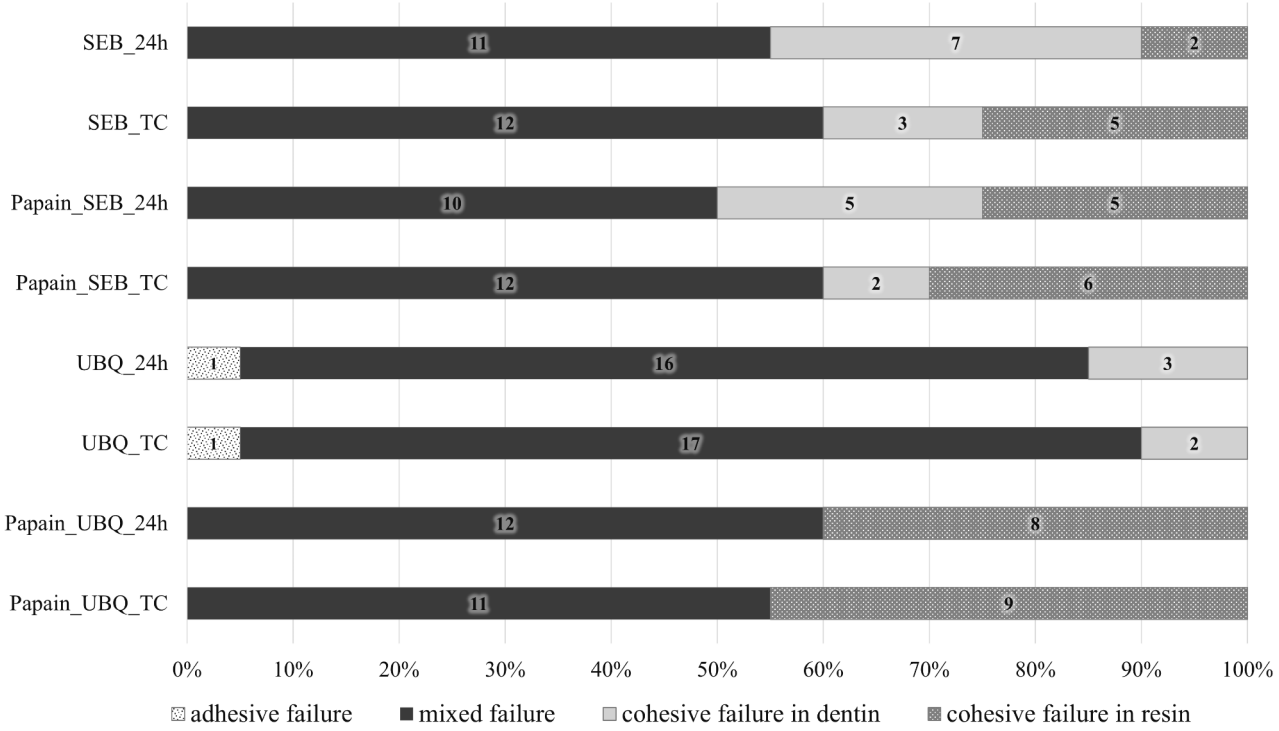

Fig. 2. Distribution of failure mode in each group. Mixed failure mode prevailed in all eroded groups Number within the bars indicate the number of specimens with the respective failure mode. Abbreviation: SEB, Clearfil SE Bond 2; UBQ, Universal bond quick; TC, 10,000 thermocycles.

Different uppercase letters indicate significant differences in each column. Different lowercase letters indicate significant differences in each row. ($p < 0.05$). Abbreviations: SEB, Clearfil SE Bond 2; UBQ, Universal Bond Quick; TC, 10,000 thermal cycles.

Fractography

Failure mode distributions for all test groups are shown in Fig. 2. SEM analysis of the fractured surfaces (Figs. 3 and 4) revealed that mixed failure was the predominant mode across all groups. Statistical analysis demonstrated significant differences in failure mode distribution between groups ($p = 0.004$). Notably, specimens bonded with UBQ showed fewer adhesive and mixed failures following papain enzyme pretreatment.

SEM observation of pretreated eroded dentin surface

SEM images of surface morphology of eroded dentin without papain enzyme pretreatment exhibited compact collagen-like structures, indicating partial demineralization at the intertubular dentin with partial occlusion of dentinal tubule (Fig. 5a). In contrast, pretreatment with papain enzyme noticeably removed the occlusion of dentinal tubules and reduced the amount of collagen-like structures in the intertubular dentin (Fig. 5b).

SEM observation of resin-dentin interface

Representative SEM images of resin-dentin interface of the UBQ groups exhibited longer resin tags compared to the SEB groups (Fig. 6). A thicker hybrid layer and longer resin tags were observed in the SEB group with papain- pretreatment (Fig. 6b) compared to the group without pretreatment (Fig. 6a). Additionally, denser and longer resin tags were observed in the UBQ group with papain pretreatment (Fig. 6d) compared to the group without pretreatment (Fig. 6c).

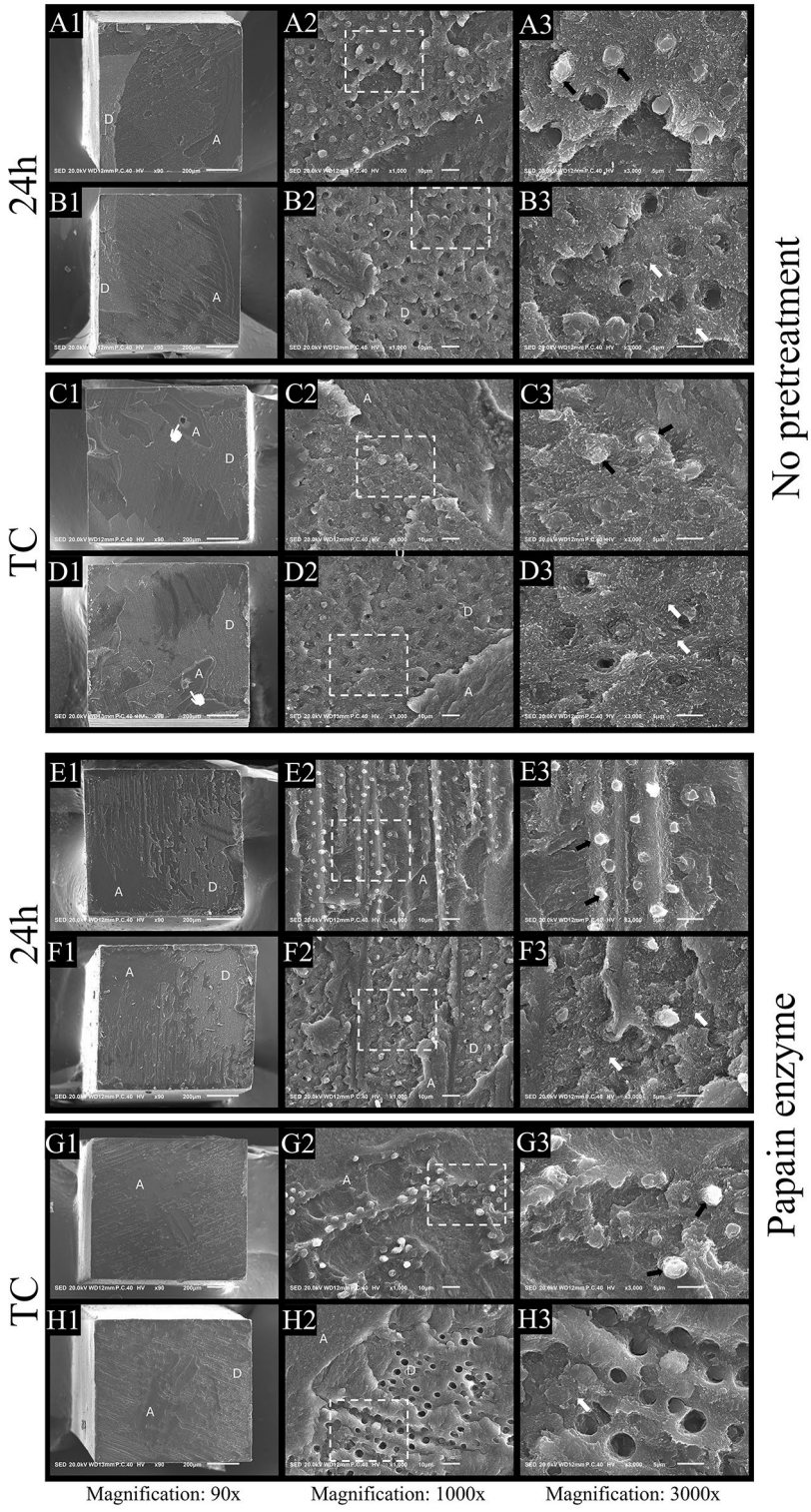

Fig. 3. Representative SEM images of fractography of Clearfil SE Bond 2. Black arrows indicate resin tags (A3, C3, E3, and G3). The compact intertubular collagen fibrils (white arrows) were clearly observed in the untreated control group (B3 and D3) compared to papain-pretreated group (F3 and H3). Bubbles (finger) were observed in the untreated control group after TC (C1 and D1). Abbreviations: A, adhesive; D, dentin; TC, 10,000 thermal cycles.

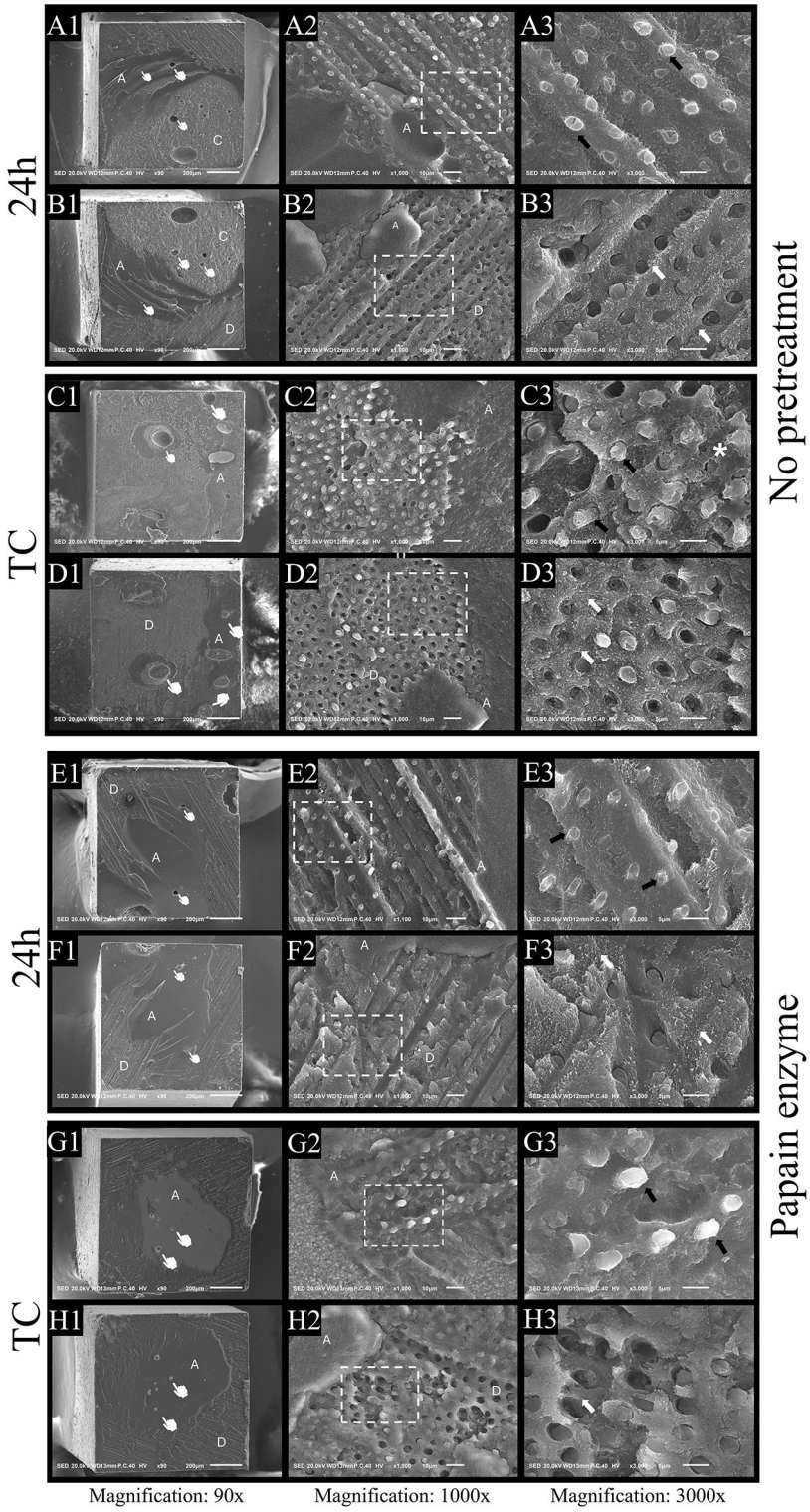

Fig. 4. Representative SEM images of fractography of Clearfil Universal Bond Quick. Black arrows indicate resin tags (A3, C3, E3 and G3). The compact intertubular collagen fibrils (white arrows) were clearly observed in the untreated control group (B3 and D3) compared to papain pretreated group (F3 and H3). Large bubbles were observed in the groups of no-pretreated control (fingers, A1, B1, C1 and D1), while small bubbles were observed in the groups of papain pretreatment (finger, E1, F1, G1 and H1). Nonhomogeneous porous adhesive layer (star, C3) was observed in untreated control group after TC. Abbreviations: A, adhesive; C, resin composite; D, dentin; TC, 10,000 thermal cycles.

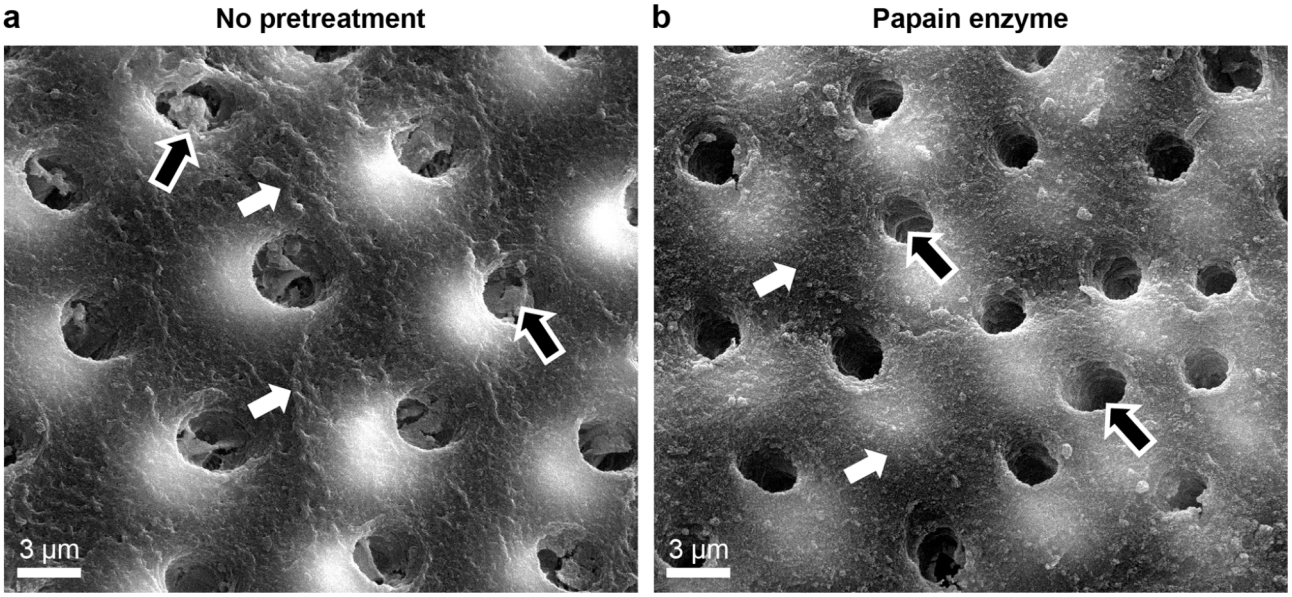

Fig. 5. Representative SEM images of treated eroded dentin surface at magnification 10,000x. Dense collagen-like structure (white arrows) and partial occluded dentinal tubule (black arrows) were observed on untreated eroded dentin surface (**a**). Lesser collagen-like structure (white-arrows) and open dentinal tubules (black arrows) were observed on the papain-pretreated eroded dentin surface (**b**).

Deproteinizing effect

The representative Raman spectra which were normalized to the phosphate stretching vibration at 960 cm^{-1} are presented in Fig. 7a. Pretreatment with papain enzyme revealed a reduction in the amide I peak at 1670 cm^{-1} (Fig. 7a), compared to the baseline spectra. The paired t-test revealed significant reduction of the amide-to-phosphate ratio after pretreatment with papain enzyme ($p < 0.001$, Fig. 7b).

Discussion

The results revealed that papain enzyme pretreatment exhibited remarkable efficacy in enhancing the bond performance of SEAs on eroded dentin surfaces. The pretreatment protocol significantly increased the initial bond strength and maintained stability following TC. Through comprehensive analysis, the study revealed multiple mechanisms underlying this improvement: significant reduction in the amide-to-phosphate ratio, favorable modification of eroded dentin ultrastructure, and enhanced resin-dentin interface characteristics, evidenced by increased hybrid layer thickness and elongated resin tags formation. Therefore, all null hypotheses were rejected.

Bonding to eroded dentin presents significant challenges due to its compromised ultrastructure, which shows mineral loss, matrix softening, and exposure of denatured collagen fibrils[38–40]. These structural changes reduce resin infiltration and weaken the chemical interaction between the adhesive monomers and the underlying dentin substrate[39,41].

Various approaches have been investigated to enhance the adhesive interface on eroded dentin, primarily focusing on either removing or stabilizing the demineralized collagen layer. Among these strategies, cross-linking agents (such as riboflavin and proanthocyanidin) and selective organic matrix removal using NaOCl and HOCl have demonstrated the promising results. However, the optimal application protocols for cross-linking agents require further investigation, while NaOCl and HOCl treatments have been shown to impair the degree of polymerization of adhesives. Papain enzyme effectively addresses these limitations through its selective proteolytic activity[26]. The enzyme provides superior benefits by dissolving denatured organic components while preserving the intact collagen network essential for bonding, without the negative effects on polymerization of adhesives. The papain enzyme utilized in this investigation, derived from green papaya and formulated at 10% concentration (3000 U/mg), demonstrates enhanced efficacy through its Encapsulated Buffer Emulsion technology[42,43]. This sophisticated delivery system optimizes the enzyme's capability to degrade partially denatured collagen molecules while maintaining structural integrity of the sound dentin matrix[42–45]. From a clinical perspective, this formulation offers a feasible chairside protocol with its reasonable 30-second application time. Previous studies have demonstrated favorable biocompatibility with pulp cells and minimal inflammatory response at this concentration[46,47], suggesting its safety for clinical application.

The dual analytical approach employing Raman spectroscopy and SEM microscopy provided compelling evidence of the enzyme's deproteinizing capability, manifested as a significant reduction in the amide-to-phosphate ratio (Fig. 7), dissolved the collagen like-structures, and improved tubular patency (Fig. 5b).

A particularly noteworthy finding is the formation of a more robust hybrid layer and increased resin tags penetration following papain pretreatment (Fig. 6b and d). While previous studies suggest that resin tags length

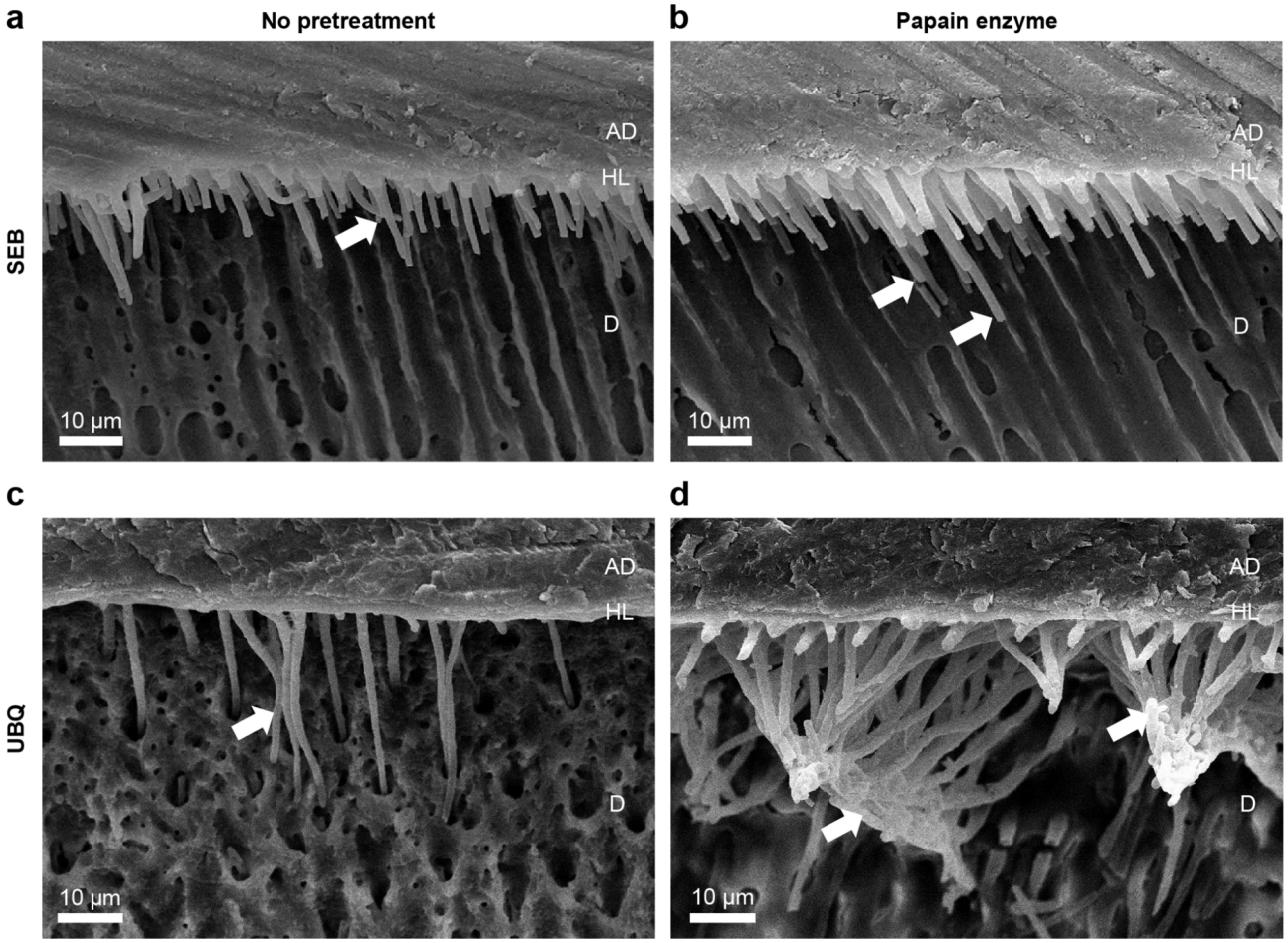

Fig. 6. Representative SEM image of resin-dentin interface at magnification 3,000x. UBQ groups showed longer resin tags than SEB groups (white arrows). Comparing to the group of SEB without treatment (**a**), the thicker HL and longer resin tags (white arrows) were observed in the group of SEB with papain pretreatment (**b**) Comparing to the group of UBQ without treatment (**c**), The thicker HL with compact resin tags were observed in the group of UBQ with papain pretreatment (**d**).Abbreviation: AD: adhesive; D: Dentin; HL: hybrid layer; SEB, Clearfil SE Bond 2; UBQ, Clearfil Universal Bond Quick.

may have minimal direct impact on bond strength[29], the enhanced hybrid layer thickness observed in this study correlates strongly with improved μTBS[48].

Both SEB and UBQ contain 10-Methacryloyloxydecyl dihydrogen phosphate (10-MDP) and fluoride. A distinctive feature of UBQ is its partial substitution of HEMA with a novel hydrophilic amide monomer, which exhibits dual characteristics - enhanced hydrophilicity before polymerization and increased hydrophobicity after polymerization[32]. This molecular design facilitates rapid infiltration, promotes high bond strength, and reduces water sorption.

Following the manufacturers' instructions, UBQ's zero-waiting application demonstrated effective penetration, evidenced by the formation of extended resin tags attributable to the properties of the novel amide monomer.

Despite these advantageous characteristics, the present study revealed significantly lower bond strength in UBQ groups compared to SEB groups, potentially attributable to application methods. Fractography SEM analysis revealed prominent water blister at the UBQ adhesive interface. This phenomenon likely occurs because eroded dentin, being partially demineralized, retains higher water content compared to normal dentin covered with smear layer. The presence of water channels and blisters becomes more pronounced with increasingly hydrophilic simplified adhesive systems[49]. Furthermore, specimens without pretreatment exhibited deterioration after thermocycling, manifesting as porous, nonhomogeneous adhesive layer. These results suggest that the single-bottle adhesive application protocol, characterized by brief application and air-drying period, may be inadequate for establishing reliable bonds to organic-rich eroded dentin. Modification to extend both application and air-drying intervals could potentially enhance the bonding effectiveness of single-bottle adhesive systems.

The erosive cycling model used in this study effectiveness simulated natural erosive challenges, producing an appropriate erosive dentin surface characterized by partially demineralized dentin and occluded dentinal tubules. While this in vitro model provided valuable insights into the mechanisms of erosion and adhesion, it represents a simplified version of the complex oral environment. Additionally, comprehensive evaluation of this

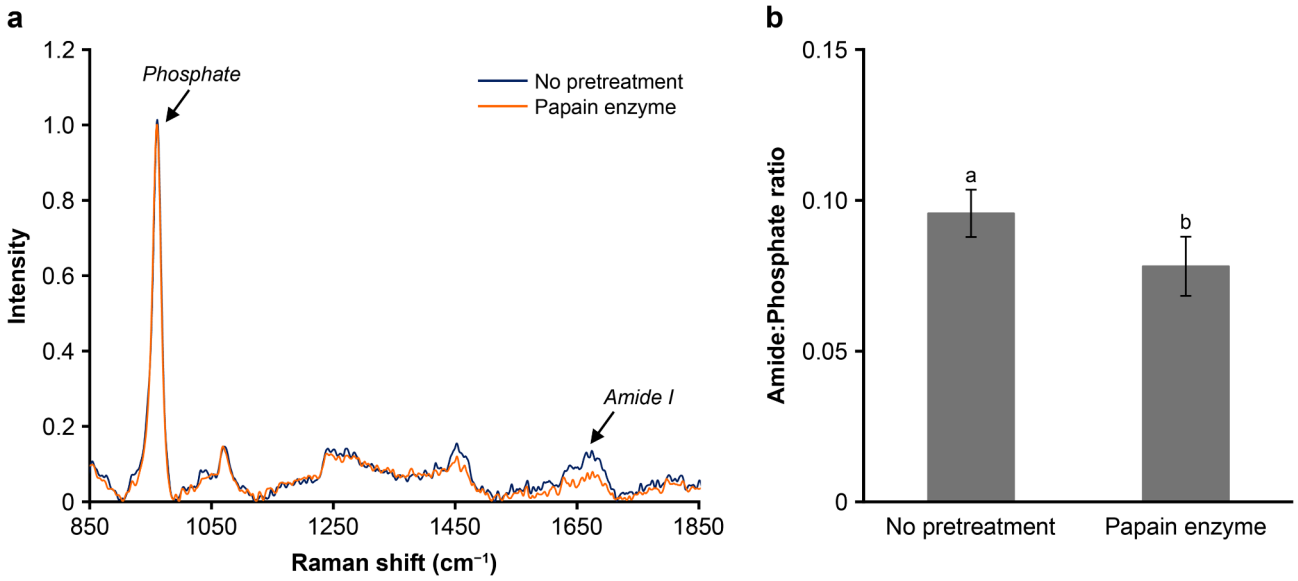

Fig. 7. Representative Raman spectra of eroded dentin surface with or without papain-pretreatment (**a**). The spectra were normalized to v1 phosphate (peak at 960 cm-1). Mean and standard deviation of amide-to-phosphate ratio of eroded dentin surface with or without pretreatment by papain enzyme (**b**). Different lowercase letters represent significant difference ($p < 0.001$).

approach with various contemporary adhesive systems and organic-rich dentin substrates would enhance our understanding of its clinical applicability. Such investigations would provide valuable insights into optimizing adhesive protocols for eroded dentin and potentially lead to improved clinical outcomes in adhesive dentistry.

Conclusion

Deproteinization with papain enzyme dissolved the organic components on eroded dentin surface, leading to the improvement of resin infiltration, increase in thickness of hybrid layer, and improvement of bond durability of SEAs to eroded dentin.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author contributions

P.S. and K.S. wrote the main manuscript text. P.S. conducted the principal investigation, developed the methodology, curated the data, performed the formal analysis, and created the visualizations. K.S. secured funding, conducted the investigation, managed the project, and supervised the work. J.T. and P.T. contributed to the conceptualization, formal analysis, methodology, project administration, and supervision, and reviewed and edited the manuscript. R.B. and T.N. conducted the investigation, provided resources, contributed to the software, created visualizations, and reviewed and edited the manuscript. C.K. conceptualized the study, developed the methodology, validated the results, and reviewed and edited the manuscript. N.H. and Y.S. provided resources, contributed to the software, and reviewed and edited the manuscript. All authors reviewed the manuscript, gave final approval, and agreed to be accountable for all aspects of the work.

Declarations

Competing interests
The authors declare no competing interests.

Research involving human participants and/or animals
The study was conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments. The use of extracted human teeth in this study was approved by the Human Research Ethics Committee of the faculty of Dentistry Chulalongkorn University under protocol number 103/2023 and 022/2024.

Informed consent
Informed consent was obtained from all subjects.

Additional information
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