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ORIGINAL ARTICLE

Evaluation of physicochemical and antibacterial properties of an experimental adhesive resin containing casein phosphopeptide-amorphous calcium phosphate

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Abstract

This study aimed to evaluate the polymerization, contact angle, surface tension, antibacterial activity, and bonding properties of experimental adhesives modified with the addition of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP). A conventional universal adhesive resin was modified by incorporating CPP-ACP at different concentrations by weight (1% and 3%), resulting in experimental adhesive resin groups. The groups were classified as Control (without CPP-ACP), 1% CPP-ACP, and 3% CPP-ACP. The microshear bond strength (μSBS) to enamel and dentin, monomer conversion degree (DC), antibacterial activity against *S. mutans* and *L. casei*, contact angle, and surface tension were evaluated. The antibacterial activity of the materials was assessed using the agar diffusion method. The obtained data were statistically analyzed, and a significance level of $p<0.05$ was considered. When comparing the DCs of the adhesive resins, no statistically significant difference was found between the Control group, 1% CPP-ACP, and 3% CPP-ACP groups ($p>0.05$). However, a statistically significant difference was observed between the 1% CPP-ACP and 3% CPP-ACP groups ($p<0.05$). According to the μSBS test results, no significant difference was observed in the μSBS values related to the tooth substrates (enamel and dentin) ($p>0.05$). When comparing the μSBS to dentin, a statistically significant difference was found between the 1% CPP-ACP group and the other groups ($p<0.05$), while no significant difference was found between the Control and 3% CPP-ACP groups ($p>0.05$). Regarding the μSBS to enamel, only a significant difference was found between the Control group and the 3% CPP-ACP group ($p<0.05$). When evaluating the antibacterial activity of the adhesive resins against *S. mutans*, an increase in antibacterial activity was observed with the increasing CPP-ACP concentration ($p<0.05$). No antibacterial activity was observed against *L. Casei*. According to the contact angle and surface tension test results, no statistically significant difference was observed between the groups ($p>0.05$). The incorporation of CPP-ACP into the universal adhesive resin influenced bonding performance in a concentration- and substrate-dependent manner, with 1% CPP-ACP enhancing dentin bond strength and 3% CPP-ACP improving enamel bonding. Clinical Significance: CPP-ACP-modified adhesives demonstrated substrate-specific benefits, with 1% CPP-ACP enhancing bond strength to dentin and 3% CPP-ACP improving adhesion to enamel. These findings suggest that tailored CPP-ACP concentrations may optimize bonding performance while providing antibacterial effects, without compromising the fundamental properties of the adhesive system.

Keywords Adhesive resin · Amorphous calcium phosphate (CPP-ACP) · Antibacterial activity · Shear bond strength · Contact angle · Monomer conversion

Introduction

Due to their superior aesthetic properties and optical characteristics resembling those of natural tooth structures, dental composite resins are widely used today for the restoration of structurally compromised teeth caused by caries [1, 2]. Composite resins adhere to the tooth structure through

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bonding systems (adhesive resins) via micromechanical and chemical interactions. This adhesive bond allows for the application of minimally invasive preparation techniques, promoting the preservation of healthy tooth tissue and enhancing biological compatibility. However, the tooth–composite interface continues to be the weakest link in the restoration complex. Over time, thermal, mechanical, and chemical stresses acting on this interface may result in the formation of microgaps, which can lead to microleakage. Such microleakage facilitates the penetration of bacteria beneath the restoration, potentially leading to the development of secondary caries [3, 4]. Therefore, the effectiveness and long-term stability of adhesive systems are critical determinants of the clinical success of restorations.

In recent years, the scope of restorative materials has expanded beyond merely providing structural integrity to also include properties that inhibit caries (anticariogenic) and promote the remineralization of dental tissues. In this context, researchers have investigated the incorporation of various bioactive agents such as fluoride, chlorhexidine, graphene oxide, calcium chloride, calcium phosphate compounds, bioactive glass, hydroxyapatite, silver nanoparticles, and quaternary ammonium compounds into restorative materials [5]. The incorporation of bioactive agents into adhesive systems has gained increasing attention as a strategy to enhance not only micromechanical bonding but also the biological stability of the tooth–adhesive interface. Although various bioactive materials, such as bioactive glasses, nano-hydroxyapatite, and antibacterial monomers, have been investigated, studies specifically evaluating the incorporation of casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) into adhesive resin matrices remain limited [5].

CPP-ACP is a biologically active remineralizing agent known for its potential to prevent dental caries [5, 6]. CPP is derived from casein, the principal phosphoprotein in bovine milk, and consists of phosphopeptide sequences that stabilize calcium and phosphate ions. These peptides form complexes with amorphous calcium phosphate (ACP), which localize to the tooth surface and help restore the calcium-phosphate balance in demineralized enamel and dentin, thereby supporting remineralization [7]. Due to these properties, CPP-ACP not only plays a preventive role when incorporated into restorative materials by inhibiting caries formation but also contributes to the repair of early enamel lesions [8]. Furthermore, CPP-ACP has been reported to exhibit antimicrobial effects on dental plaque and may reduce the growth and surface adhesion of cariogenic bacteria, particularly *Streptococcus mutans* [9]. CPP-ACP is distinguished from other bioactive agents by its ability to stabilize calcium and phosphate ions and form a bioavailable ionic reservoir on enamel and dentin surfaces. While its remineralizing and antibacterial effects have been extensively demonstrated when applied as a surface treatment, the clinical effectiveness of this approach is largely dependent on the type and frequency of application, and it should be noted that the procedure can be time-consuming. In contrast, direct incorporation of CPP-ACP into the adhesive resin matrix may provide a localized and sustained bioactive effect at the bonding interface without the need for an additional clinical step, potentially improving clinical applicability.

This study aimed to comprehensively evaluate a novel material developed by incorporating CPP-ACP, a bioactive agent that has been scarcely investigated in the literature, into an experimental bonding agent, in terms of its polymerization behavior, wettability characteristics (contact angle and surface tension), antibacterial activity, and bonding capacity to dental tissues (dentin and enamel). Through this comprehensive evaluation, the study seeks to provide new insights into the development of bioactive adhesive systems that may contribute to enhanced clinical performance and caries prevention. The null hypotheses of this study were that the incorporation of CPP-ACP into the adhesive resin would not affect (1) its antibacterial activity, (2) degree of polymerization, (3) micro-shear bond strength, or (4) surface properties, including contact angle and surface tension.

Materials and methods

The research protocol of this study was approved by the Erciyes University Clinical Research Ethics Committee (App No: 2023/546). In this study, experimental adhesive resins were formulated by incorporating different concentrations of CPP-ACP into a conventional universal adhesive resin. The physical, chemical, and biological properties of these experimental adhesives, including degree of monomer conversion, bond strength to enamel and dentin, antibacterial activity, contact angle, and surface tension, were comparatively evaluated against the conventional universal adhesive resin. The overall experimental design, group allocation, and testing sequence are summarized in a schematic diagram (Fig. 1).

Synthesis of CPP-ACP

A 10% (w/v) casein solution was prepared at pH 8 (adjusted using NaOH). Trypsin was then added to the solution at 0.2% (w/w) relative to casein, and the mixture was stirred at 50 °C for 2 h. The pH of the solution was adjusted to 4.6 by the addition of HCl. Following this, the solution was centrifuged, and the pH of the resulting precipitate was adjusted to 9 using NaOH. To achieve final concentrations

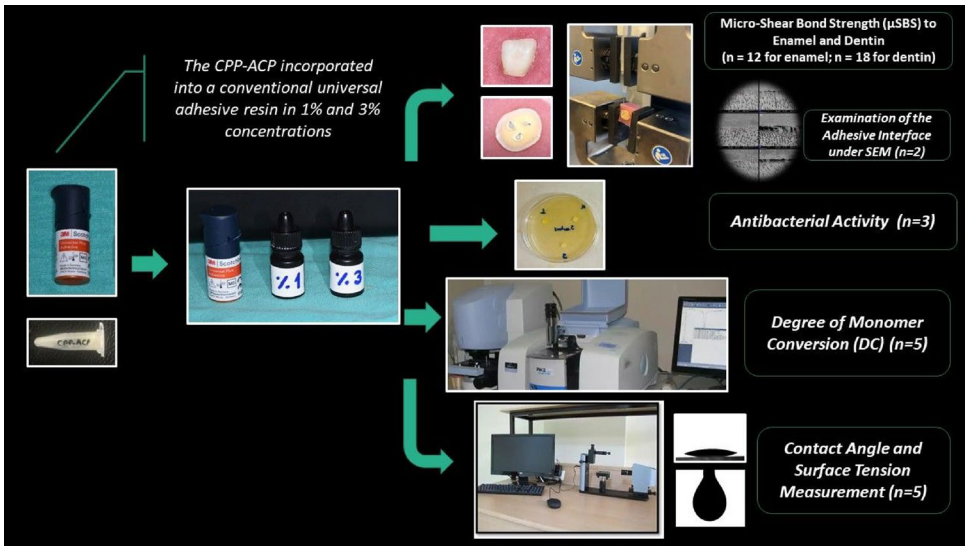


Fig. 1 Schematic illustration of the experimental design

of calcium chloride (100 mM Ca^{2+}), sodium phosphate (60 mM phosphate), and sodium fluoride (12 mM fluoride), $CaCl_2$ (1 mol), Na_2HPO_4 (1 mol), and NaF (200 mmol) were slowly added to the solution. The solution was filtered using a microfilter (0.1–0.2 µm), and the resulting precipitate was dried. The final powder contained approximately 50% CPP and 40% ACP [10]. Characterization of the synthesized CPP-ACP powder was performed using SEM, XRD, FTIR, and EDX analyses to confirm its morphology, crystallinity, bonding structure, and composition.

Incorporation of CPP-ACP into adhesive resin and determination of study groups

Following the synthesis of CPP-ACP, the obtained powder was incorporated into a universal adhesive resin (Scotchbond Universal Plus, 3 M ESPE, St. Paul, MN, USA) at two different concentrations (1% and 3% by weight (w/w)). After accurately measuring the CPP-ACP powder for each group, the adhesive resins were dispensed into their respective bottles in a dark environment using a micropipette, and the CPP-ACP powder was added to each resin. The mixture was homogenized by shaking for 20 min at 500 rpm using a mechanical shaker (KS 125 Basic, IKA Labortechnik). Furthermore, the experimental adhesives were manually shaken for 1 min in orbital motion before each use. The study groups were defined as follows:

1. Conventional adhesive resin without CPP-ACP (Control).
2. 1% CPP-ACP containing adhesive resin (1% CPP-ACP).
3. 3% CPP-ACP containing adhesive resin (3% CPP-ACP).

Evaluation of antibacterial activity of the experimental adhesive resins

The antibacterial activity of adhesive resins with and without CPP-ACP was evaluated against *S. mutans* (ATCC 25175) and *L. casei* (ATCC 393) bacterial strains using the agar diffusion method. The bacteria were seeded onto FAA (Fastidious Anaerobe Agar with 5% Horse Blood) plates. Pre-cultures were prepared to achieve bacterial test suspensions at a concentration of 6×10^5 colony-forming units (CFU)/ml. A 150 µl bacterial suspension was evenly spread on the FAA agar plates using sterile swabs. The samples were impregnated onto sterile carrier disks using a micropipette, with 20 µl of each sample per disk. These disks were then placed onto the inoculated agar surface under aseptic conditions. The plates were incubated anaerobically at 37 °C for 48 h. After incubation, the inhibition zones formed around the disks were measured. The study was performed with three replicates for each group.

Evaluation of degree of monomer conversion (DC)

In our study, the DCs of both polymerized and unpolymerized adhesive resins ($n=5$) were measured using Fourier Transform Infrared Spectroscopy (FT-IR, PerkinElmer 400 FT-IR/FT-FIR Spectrometer Spotlight 400 Imaging System, PerkinElmer, Waltham, MA, USA). For polymerized samples, two drops of each adhesive resin were placed into a Teflon mold with a height of 0.8 mm and a diameter of 6 mm and dried with air for 10 s to allow the solvent to evaporate. After protecting the samples from oxygen with a glass cover, the polymerization process was carried out using an LED light source for 20 s. For unpolymerized samples, each adhesive resin was dropped onto the ATR

platform, and the platform and measurement tip were wiped with ethanol after each measurement.

The absorption spectra of the samples placed in the device holder were recorded before and after polymerization. Spectral measurements were carried out in the range of 400 to 4000 cm^{-1}. A reference measurement was first taken, followed by a total of 20 scans at a resolution of 4 cm^{-1} for each sample. The changes in the peak intensities of the aromatic double bonds around the 1608 cm^{-1} wavelength and the aliphatic double bonds around the 1638 cm^{-1} wavelength for polymerized and unpolymerized samples were calculated with the following formula:

$$DC\ (\%) = \left(1 - \frac{(1638\ cm^{-1}/1608\ cm^{-1})\ after\ curing}{(1638\ cm^{-1}/1608\ cm^{-1})\ before\ curing}\right) \times 100$$

Micro-shear bond strength (μSBS) to enamel and dentin

Preparation of the tooth surfaces

In this study, 18 upper central incisors (for μSBS to enamel) and 18 lower third molars (for μSBS to dentin), which were free from caries, fractures, or cracks and had not undergone root canal treatment, were used. They were stored in a 0.5% chloramine T solution at 4 °C for one week for disinfection after extraction. They were then kept in distilled water at 37 °C until use.

For testing μSBS to dentin, the occlusal enamel surfaces of the lower third molars were carefully removed using a low-speed cutting device (Isomet 1000, Buehler, Lake Bluff, IL, USA) under continuous water cooling. For testing μSBS to enamel, the upper incisor crowns were separated from the roots using a water-cooled low-speed cutting machine (Isomet 1000, Buehler, Lake Bluff, IL, USA). The teeth were embedded in acrylic resin, with occlusal dentin surfaces exposed in molars and buccal enamel surfaces exposed in incisors. The tooth surfaces were sequentially abraded using silicon carbide papers (600, 800, and 1200 grit) to create flat bonding surfaces with a standard smear layer. The surfaces of the teeth were examined under a dental loupe (Heine Binocular Loupe, Germany) at 4.5x magnification to check for the presence of enamel on molars and dentin exposure on incisors. The teeth were then randomly divided into three groups based on tested adhesive resins (Control, 1% CPP-ACP, and 3% CPP-ACP) ($n=6$).

Bonding of composite resin samples to the tooth surfaces

The adhesive resins were applied to the dentin and enamel surfaces in only self-etch mode, using an applicator for 20 s. After 5 s of application of pressured air, the resin was polymerized for 20 s using an LED light device (Valo Cordless, 1000 mW/cm², Ultradent, South Jordan, UT, USA). After the adhesive application, nanohybrid composite resin cylinders (Filtek Z550, 3 M ESPE, St. Paul, MN, USA) with a diameter of 1 mm and a height of 2 mm were attached to the tooth surfaces using tygon tubes and polymerized with the LED device for 20 s. The samples were then stored in distilled water at 37 °C for 24 h.

A power analysis indicated that the sample size for each group should be at least twelve (power = 0.80, effect size = 0.551, significance level $\alpha = 0.05$). Therefore, two composite resin cylinders were bonded to the enamel surface of each incisor and three composite resin cylinders were bonded to the dentin surface of each third molar, with at least 12 samples in each group ($n=12$ for enamel; $n=18$ for dentin).

Micro-shear bond strength (μSBS) test

In this study, the μSBS test was conducted using a universal testing machine (Instron, Model 4444; Instron Corp., Canton, USA). A 0.25 mm-thick wire ring was positioned parallel to the bonding interface and placed around the blocks in a semicircular shape, and force was applied at a rate of 1 mm/min until fracture occurred. The maximum force at the moment of fracture was recorded in Newtons (N) and divided by the bonding surface area to calculate the value in megapascals (MPa). The fracture surfaces were examined under a stereomicroscope (Stemi 1000, Zeiss, Germany) at 25× magnification to identify the modes of failure. Failure types were categorized as follows: adhesive failure — occurring at the adhesive–dentin/enamel interface; cohesive failure— occurring within the adhesive, enamel, or dentin; and mixed failure —involving both adhesive and cohesive characteristics.

Examination of the adhesive interface

For the evaluation of the adhesive interface, flat dentin surfaces were prepared from six extracted human molar teeth. The tested adhesive resins were applied to two teeth per group ($n=2$) in accordance with the previously outlined protocol. Following the adhesive application, composite resin build-up was performed in two layers, each not exceeding 2 mm. The samples were stored in a 100% humid environment at 37 °C for 24 h. Subsequently, to expose the adhesive interface, serial Sect. 2 mm thick were obtained from the samples perpendicular to the bonding plane. Two central sections from each tooth were used for analysis and mounted on acrylic resin trays, leaving the bonding interface

exposed. The section surfaces were polished using abrasive discs and diamond polishing paste.

The sample surfaces were ultrasonically cleaned for 3 min, then demineralized with 6 N HCl for 1 min. After rinsing with distilled water, the surfaces were exposed to 5% NaOCl solution for 3 min, rinsed again with distilled water, and dehydrated sequentially with 70%, 80%, 90%, and 99% ethanol.

The prepared samples were examined using a field emission scanning electron microscope (FESEM) (GeminiSEM 500, Zeiss, Oberkochen, Germany). Prior to imaging, the sample surfaces were sputter-coated with a gold-palladium (Au-Pd) alloy at a deposition rate of 3 Å/s for 15 s, resulting in a total coating thickness of 45 Å. FESEM imaging was performed at an accelerating voltage of 3.0 kV. The adhesive interfaces were thoroughly examined at 1000× magnification for the micromorphological features of the hybrid layer and resin tags.

Contact angle measurement

The contact angles of the adhesive resins were measured using an optical tensiometer device (Attension Theta Lite 101, Biolin Scientific, Gothenburg, Sweden). Samples from the Control, 1% CPP-ACP, and 3% CPP-ACP groups were dropped onto a lamellar surface. The contact angle measurements of each group were made after a 10-second waiting period using the "sessile drop method." The droplet volume for each group was standardized to 10 µl, and three measurements were taken for each sample. The contact angle (θ) values were then recorded.

Surface tension measurement

Surface tension measurements of the Control, 1% CPP-ACP, and 3% CPP-ACP groups (with a 10 µl droplet volume) were performed using the "hanging drop method" with an optical tensiometer device (Attension Theta Lite 101, Biolin Scientific, Gothenburg, Sweden). Drop images were captured, and drop shape analysis was performed using the corresponding software package (Attension Theta Software, Biolin Scientific, Stockholm, Sweden). The surface energies of the adhesive resins were calculated using the Young-Laplace equation. Three measurements were taken for each sample.

Table 1 Mean inhibition zone diameters (mm) and standard deviations (SD) of the adhesive resins against Streptococcus mutans ($n=3$)

Groups	Mean ± SD	Median (Min-Max)	Test statistics	p
Control	9.75 ± 0.5	10 (9–10)[a]	8.171	**0.017**[x]
1% CPP-ACP	12.5 ± 1.732	12.5 (11–14)[ab]		
3% CPP-ACP	16.75 ± 4.992	16.5 (12–22)[b]		

[x]Kruskal–Wallis H test

[a-b]Groups with the same letter are not significantly different

Statistical analysis

Data were analyzed using IBM SPSS V23 and Jamovi V2.3.28. The normality of the data was assessed using the Shapiro-Wilk test to determine the appropriate statistical approach. For normally distributed data, one-way analysis of variance (ANOVA) was applied to compare group means, with multiple comparisons performed using the Tukey test. For non-normally distributed data, the Kruskal-Wallis H test was used, followed by the Dunn test for pairwise comparisons. To account for both group and tooth surface effects in non-normally distributed data, Robust ANOVA was performed using the Walrus package, with Bonferroni adjustment for multiple comparisons. These tests were selected to ensure appropriate handling of data distribution and multiple comparison correction. The significance level was set at $p<0.05$.

Results

Antibacterial activity

When the antibacterial activity of adhesive resin without CPP-ACP, 1% and 3% CPP-ACP-containing experimental adhesive resins was evaluated, it was observed that no inhibition zone was formed against *L. casei* in any of the three variations. In contrast, a statistically significant difference in antibacterial activity against S. mutans was detected among the groups ($p=0.017$). The highest antibacterial activity was observed in the 3% CPP-ACP group, followed by the 1% CPP-ACP group. While the 3% CPP-ACP group showed significantly greater antibacterial activity than the control group ($p<0.05$), no significant differences were found between the control and 1% CPP-ACP groups or between the 1% and 3% CPP-ACP groups ($p>0.05$).The mean inhibition zone diameters and standard deviations formed by the adhesive resins against *S. mutans* are presented in Table 1.

Degree of monomer conversion

The average degree of monomer conversion of the tested adhesive resins, standard deviations, and statistical differences are presented in Table 2. There was a statistically significant difference among the groups regarding monomer conversion ($p=0.019$). The 3% CPP-ACP group showed the highest degree of monomer conversion (55.10%), followed by the Control group (52.38%). The differences between the

Table 2 Mean degree of monomer conversion (%) of the tested adhesive resins ($n=5$), standard deviations (SD), and statistical differences

Groups	Mean ± SD	Median (Min-Max)	Test statistics	p
Control	52.38 ± 2.57[ab]	51.34 (49.44–55.24)	8.627	**0.019**[x]
1% CPP-ACP	50.16 ± 0.49[a]	50.39 (49.46–50.6)		
3% CPP-ACP	55.10 ± 2.7[b]	54.49 (52.55–58.62)		

[x]One-way analysis of variance (ANOVA)

[a-b]No significant difference was found between groups sharing the same letter (p > 0.05)

Control group and both the 1% and 3% CPP-ACP groups were not statistically significant ($p>0.05$), while a statistically significant difference was observed between the 1% CPP-ACP group and the 3% CPP-ACP group ($p<0.05$).

Microshear bond strength

The mean μSBSs of the adhesive resins on enamel and dentin surfaces and standard deviations are shown in Table 3; Fig. 2. When bond strength was analyzed irrespective of the bonded substrate, a statistically significant difference was observed among the groups ($p=0.031$). The 1% CPP-ACP group showed the highest mean bond strength (21.67 MPa), followed by the 3% CPP-ACP group (19.71 MPa) and the Control group (17.47 MPa). The Control group differed significantly from both CPP-ACP groups ($p<0.05$), whereas no significant difference was found between the 1% and 3% CPP-ACP groups ($p>0.05$).

When the bond strength values were evaluated according to the bonded substrate regardless of the adhesive resin, no statistically significant difference was found between the bond strength values to enamel (19.83 MPa) and dentin (18.48 MPa) ($p=0.400$).

Comparison based on both group and tooth tissue revealed a statistically significant difference in bond strength values ($p=0.002$). Analysis of enamel and dentin bond strengths within each group showed no statistically significant difference in the Control and 1% CPP-ACP groups ($p>0.05$), but a significant difference was detected in the 3% CPP-ACP group ($p<0.05$).

In addition, in terms of dentin bond strength, a statistically significant difference was found between the 1% CPP-ACP group (22.32 MPa) and the other groups ($p<0.05$), while no difference was found between the Control group (17.48 MPa) and the 3% CPP-ACP group (16.66 MPa) ($p>0.05$).

Regarding enamel bond strength, a statistically significant difference was found between the Control group (17.35 MPa) and the 3% CPP-ACP group (22.44 MPa) ($p<0.05$), while no statistically significant difference was observed between the 1% CPP-ACP group (19.35 MPa) and the other groups ($p>0.05$).

Analysis of failure modes following the μSBS test revealed a predominance of adhesive failures in both enamel and dentin specimens, with no statistically significant differences among the groups ($p>0.05$). The distribution of failure types is summarized in Table 4.The bonding interfaces corresponding to each adhesive resin are presented in the FE-SEM micrographs shown in Figs. 3 and 4. Morphological analysis of these images demonstrated that the resin tags in the 1% CPP-ACP group exhibited greater density and length compared to those in the control group and, notably, the 3% CPP-ACP group.

Table 3 Mean microshear bond strength (μSBS, MPa) values of the adhesive resins and standard deviations ($n=12$ for enamel; $n=18$ for dentin)

Dental Substrate (DS)	Adhesive Resins (AR)			Total		p*
	Control	1% CPP-ACP	3% CPP-ACP			
Dentin	17.48 (4.86)[a]	22.32 (4.965)[b]	16.66 (8.04)[a]	18.48 (8.04)	**AR**	0.031
Enamel	17.35 (4.80)[a]	19.35 (6.96)[ab]	22.44 (6.02)[b]	19.83 (7.29)	**DS**	0.4
Total	17.47 (5.08)[a]	21.67 (7.015)[b]	19.71 (8.78)[b]	19.335 (8.895)	**AR*DS**	0.002

*Robust ANOVA was performed using the median comparison method. Data are presented as median (standard deviation)

[a–b]No significant difference between groups sharing the same letter



Fig. 2 Graphical representation of the microshear bond strength (μSBS) values of the tested adhesive resins to enamel and dentin

Table 4 Distribution of failure types (%) observed in enamel and dentin specimens

Groups	Adhesive (%)	Cohesive (%)	Mixed (%)
Enamel			
Control	9 (75)	1 (8.3)	2 (16.7)
1% CPP-ACP	10 (83.3)	0	2 (16.7)
3% CPP-ACP	10 (83.3)	1 (8.3)	1 (8.3)
Dentin			
Control	17 (94.4)	1 (5.6)	0
1% CPP-ACP	17 (94.4)	0	1 (5.6)
3% CPP-ACP	16 (88.9)	0	2 (11.1)

Contact angle

The mean contact angle measurements of the tested adhesive resins are presented in Table 5. The mean water contact angle was 28°, whereas the mean contact angles of the Control, 1% CPP-ACP, and 3% CPP-ACP groups were 24°, 20°, and 22°, respectively. No statistically significant differences were observed among the adhesive resins ($p > 0.05$).

Surface tension

The mean surface tension values and statistical differences of the tested adhesive resins are presented in Table 6. While the average surface tension measurement of water was 70 mN/m, the mean surface tension values of the Control, 1% CPP-ACP, and 3% CPP-ACP groups were found to be 28, 26, and 27 mN/m, respectively. No statistically significant difference was observed among the the adhesive resins ($p > 0.05$).

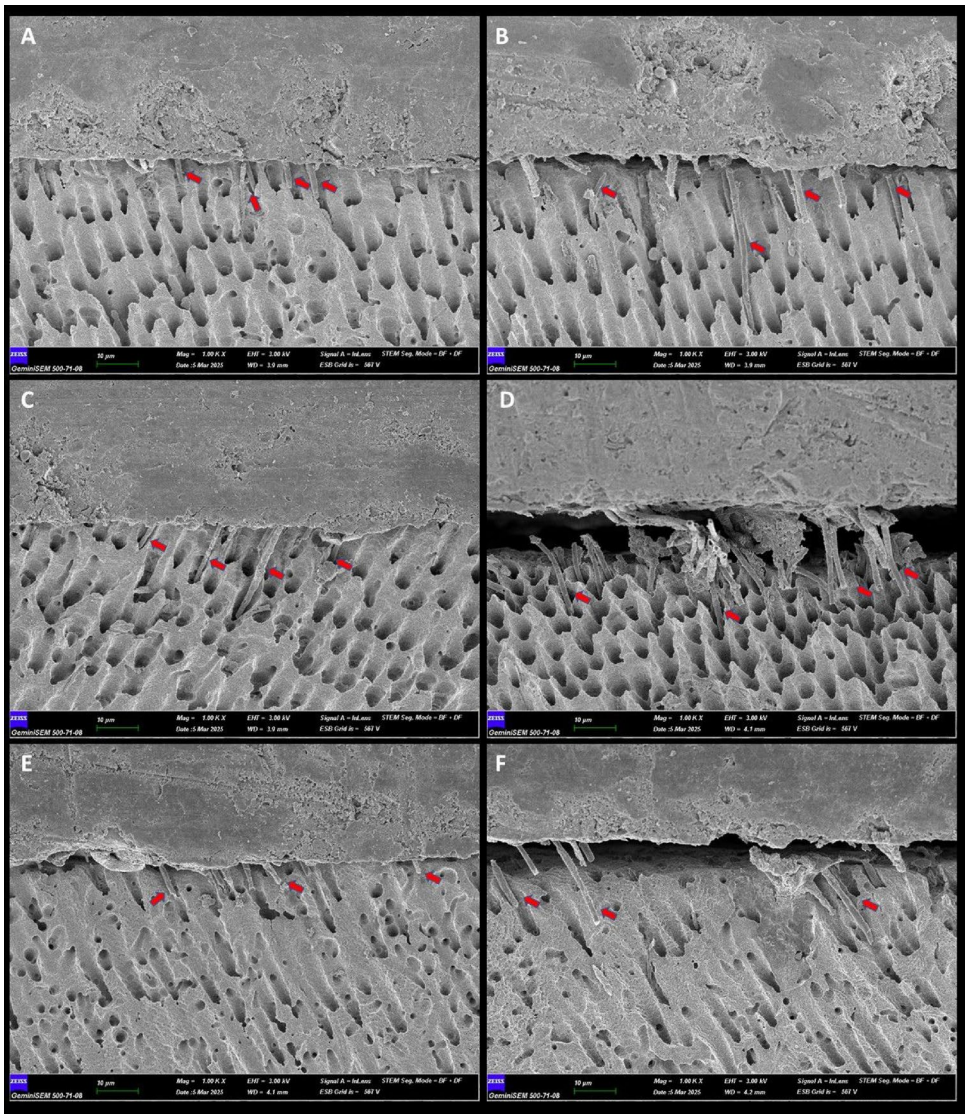


Fig. 3 FE-SEM micrographs (1000× magnification) showing the dentin bonding interfaces of the adhesive resins: Control (**A**, **B**), 1% CPP-ACP (**C**, **D**), and 3% CPP-ACP (**E**, **F**). Arrows indicate resin tags

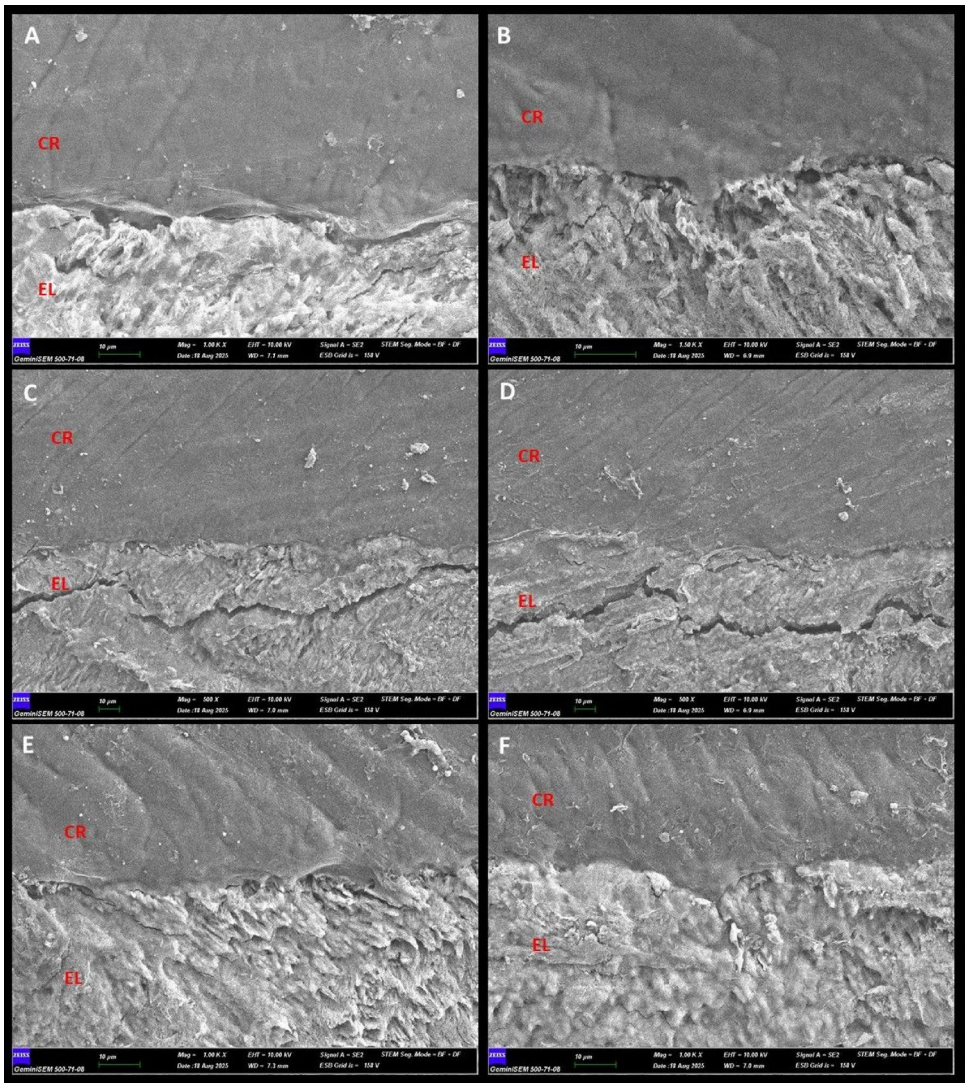


Fig. 4 FE-SEM micrographs (500× and 1000× magnification) showing the enamel bonding interfaces of the adhesive resins: Control (**A**, **B**), 1% CPP-ACP (**C**, **D**), and 3% CPP-ACP (**E**, **F**). *CR* Composite resin*; EL* Enamel

Table 5 Comparison of the contact angle values (°) of the tested adhesive resins ($n=5$)

Groups	Median (Min-Max)	Total	Test statistics	p
Water	28 (28–28)[a]	23 (20–28)	10.422	**0.015**[x]
Control	24 (23–25)[ab]			
1% CPP-ACP	20 (20–21)[b]			
3% CPP-ACP	22 (22–23)[ab]			

[x]Kruskal–Wallis H test

[a-b]Groups with the same letter are not significantly different

Table 6 Comparison of the surface tension values (mN/m) of the tested adhesive resins ($n=5$)

Groups	Median (Min-Max)	Total	Test statistics	p
Water	70 (70–70)[a]	27.5 (26–70)	9.465	**0.024**[x]
Control	28 (27–29)[ab]			
1% CPP-ACP	26 (26–26)[b]			
3% CPP-ACP	27 (26–28)[ab]			

[x]Kruskal–Wallis H test

[a-b]Groups with the same letter are not significantly different

Discussion

In this study, experimental adhesive resins were formulated by incorporating casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) at different concentrations into a conventional universal adhesive system, and their physicochemical and biological properties were evaluated. CPP-ACP is a complex formed by the stabilization of amorphous calcium phosphate through phosphopeptides derived from casein, and its remineralization-promoting effect has been well documented in the scientific literature [11]. This complex exhibits high affinity for tooth surfaces, enabling the localized release of calcium and phosphate ions. These ions contribute to the restoration of enamel and dentin structures by supporting the reformation of hydroxyapatite crystals [7, 8].

In addition to its remineralizing effects, CPP-ACP has also attracted attention for its antibacterial potential. CPP-ACP can inhibit plaque biofilm formation and reduce the

adhesion and proliferation of cariogenic microorganisms such as *Streptococcus mutans* [9]. This effect is attributed to the disruption of bacterial metabolism by calcium ions and the buffering of plaque pH, which suppresses the acidic environment. In the present study, antibacterial activity assays demonstrated that adhesive resins incorporating CPP-ACP formed distinct inhibition zones against *S. mutans*. However, to date, there are no published experimental studies directly assessing the antibacterial properties of the specific adhesive resin used in our study (Scotchbond Universal) against *S. mutans*. Nevertheless, research on the antibacterial properties of similar universal adhesives has revealed that some adhesives exhibit varying degrees of antibacterial efficacy against *S. mutans* [12]. It is hypothesized that acidic monomers, which enhance the adhesion properties of dental adhesives, contribute to the antibacterial effects observed in the adhesive resins to which they are added [13]. Furthermore, numerous studies have shown that universal adhesives can be modified with various antimicrobial agents to augment their antibacterial properties, and such modifications have been proven to inhibit the growth of *S. mutans* [14]. In a study by Chen et al., adhesive resins containing amorphous calcium phosphate nanoparticles were found to exhibit significant antibacterial activity [15]. Additionally, it was emphasized that these nanoparticles contributed to the inhibition of biofilm formation and supported the remineralization process [15].

Upon reviewing the results of the antibacterial activity tests in our study, it was observed that the universal adhesive group without CPP-ACP also formed an inhibition zone against *S. mutans*, which aligns with prior findings. Moreover, the incorporation of CPP-ACP into the adhesive resin enhanced its antibacterial activity against *S. mutans*, leading to the rejection of the first null hypothesis of our study: "The addition of CPP-ACP to the adhesive resin does not alter the material's antibacterial activity." Further research supporting our findings has shown that CPP-ACP decreases the colony-forming units of highly cariogenic bacteria such as *S. mutans* and inhibits *S. mutans* adhesion to tooth surfaces [9, 16, 17]. The antibacterial activity of the CPP-ACP-containing adhesive resin against *S. mutans* is likely the result of multiple interconnected mechanisms. The CPP-ACP (casein phosphopeptide–amorphous calcium phosphate) delivers bioavailable calcium and phosphate ions to the local environment. These ions can buffer local acidity, thereby interfering with the growth conditions essential for *S. mutans*, which are known to be highly acidogenic and aciduric microorganisms [18]. Specifically, the release of calcium and phosphate ions may disrupt the acidic microenvironment that supports *S. mutans'* survival and metabolic activity.

While our study observed antibacterial activity against *S. mutans*, no similar effect was noted against *L. casei*. In contrast to our findings, Dashper et al. concluded in their studies that treatment with casein phosphopeptide-amorphous calcium phosphate resulted in a reduction of *L. casei* biofilm [19]. In their study, CPP-ACP was applied at a 2% concentration directly onto enamel surfaces in its pure form, without being incorporated into any material, and was evaluated over a period of four days. However, the study did not provide detailed information regarding the production and synthesis of CPP-ACP, nor the form in which it was used. In contrast, our study incorporated CPP-ACP into the adhesive resin rather than applied as a surface treatment, at concentrations up to 3%; however, the amount of CPP-ACP that may be released from the resin to exert an antibacterial effect remains unknown. Thus, one potential explanation for the discrepancies between the studies may lie in the purity and formulation differences of the CPP-ACP used. It is conceivable that in the previous study, a minimum inhibitory concentration for *L. casei* was reached. Additionally, significant methodological differences exist between the studies; our study employed the agar diffusion method, while the previous study utilized DNA extraction techniques.

A literature review reveals that the monomer conversion degree of adhesive resins generally ranges from 35% to 77%. Hasegawa et al. reported that the monomer conversion degree of Scotchbond Universal adhesive ranged from 42% to 54% [20]. In our study, the average monomer conversion degree of the Control group, which did not contain CPP-ACP, was found to be 52.38%, while the average monomer conversion values for the 1% and 3% CPP-ACP groups were 50.16% and 55.10%, respectively. No statistically significant differences were observed between the Control and CPP-ACP-modified adhesives. Accordingly, the second null hypothesis, stating that "the addition of CPP-ACP to the adhesive resin does not alter the polymerization level of the material," is generally supported, although a concentration-dependent trend was noted, with the 3% CPP-ACP group showing slightly higher conversion, suggesting a possible effect of higher CPP-ACP concentrations on monomer-to-polymer conversion.

The polymerization process occurs through the initiation and propagation of chain reactions mediated by free radicals interacting with monomers. The calcium and phosphate ions present in CPP-ACP may interact with free radicals, potentially influencing their stability and modulating polymerization-related chain reactions [21, 22]. A 1% concentration of CPP-ACP may not provide sufficient ion availability in the reaction medium, which could limit these interactions. In contrast, a 3% concentration may provide greater ion availability, which might contribute to improved radical stabilization and a higher monomer-to-polymer conversion rate.

However, these interpretations should be considered with caution, as ion release was not directly evaluated in the present study. Furthermore, previous studies have reported a direct correlation between the viscosity of adhesive systems and their degree of conversion [21]. At higher viscosity levels, the mobility of polymer radicals is reduced, leading to fewer chain terminations. This "gel effect" increases free radical propagation and vinyl conversion, ultimately accelerating the rate of polymerization [21, 22]. In addition, the degree of monomer conversion in resin materials can also be influenced by several formulation-related factors, including monomer content, type, and amount of inorganic fillers, as well as the quantity and nature of solvents used [23, 24]. In light of this information, the increase in monomer conversion observed in our study following the addition of 3% CPP-ACP may be attributed to a rise in the viscosity of the adhesive resin. Moreover, the highly hydrophilic CPP-ACP nanocomplex may have absorbed some of the solvents present in the traditional adhesive resin used in the study, resulting in a reduced overall solvent content and thus contributing to improved polymerization. Nanofillers can also enhance light scattering within the adhesive matrix, thereby increasing the activation of photoinitiators [25]. This facilitates deeper light penetration and improves the degree of polymerization.

Upon comparison of the dentin bond strengths among the tested adhesive resins, the 1% CPP-ACP group exhibited a statistically significant difference compared to the other groups, whereas no significant difference was observed between the Control group and the 3% CPP-ACP group. In terms of enamel bonding, a significant difference was found between the Control and 3% CPP-ACP groups, whereas the 1% CPP-ACP group was found to be statistically similar to both. Based on these findings, it can be concluded that the μSBS varied depending on the CPP-ACP concentration and the type of tooth substrate. Therefore, the third null hypothesis, "The addition of CPP-ACP to the adhesive resin does not affect the micro-shear bond strength of the material", was rejected.

From a bonding perspective, enamel provides a reliable and predictable substrate for adhesion due to its highly mineralized and homogeneous structure. In contrast, dentin presents significant challenges because of its tubular architecture, higher organic content, and structural heterogeneity. Achieving effective bonding to dentin requires sufficient infiltration of the adhesive system into the demineralized collagen matrix and maintaining the long-term stability of the resin–dentin interface [26, 27]. This process is a critical step that directly influences the effectiveness of both micromechanical retention and chemical adhesion. Therefore, the differing bond strength outcomes of adhesives containing varying concentrations of CPP-ACP on enamel and dentin may be attributed to the distinct structural and chemical characteristics of these two dental substrates.

Several studies in the literature have reported that the application of CPP-ACP to the tooth surface can significantly enhance the bond strength of adhesive resins [28, 29]. Sattabanasuk et al. suggested that the application of CPP-ACP may increase chemical interactions between calcium ions and the functional monomers of adhesives to some extent, which could be beneficial for improving adhesion to dentin [30]. However, it should be emphasized that these studies primarily evaluated CPP-ACP as a surface treatment, rather than as a component incorporated directly into the adhesive resin. To date, only one experimental study has investigated the direct incorporation of CPP-ACP into adhesive resin systems [31]. Yeganeh et al., in their study evaluating the bond strength and remineralization potential of CPP-ACP-containing adhesive systems, concluded that while remineralization increased, no significant difference was observed in dentin bond strength [31].

Modifications in adhesive formulations influence their mechanical and physical properties, thereby affecting overall bond performance. Effective adhesion ensures interfacial stability, contributes to long-term bond durability, and is critical for the success of restorative treatments. Bond strength is largely determined by the chemical composition of the adhesive, including the structure of functional acidic monomers, solvent balance, and filler content, which govern flow behavior, dentin infiltration, and hybrid layer formation [32]. Composed mainly of monomers, solvents, and photoinitiators, adhesives must achieve adequate wetting of demineralized surfaces, penetration into dentinal tubules and the collagen matrix, copolymerization within the oxygen-inhibited layer, and micromechanical or ionic interactions with hydroxyapatite [33]. Therefore, the adhesive's ability to spread and wet the substrate is a fundamental factor influencing bond quality. In our study, an increase in CPP-ACP concentration from 1% to 3% was associated with enhanced bond strength to enamel, whereas a decrease in bond strength was observed in dentin. This reduction in dentin bond strength at 3% CPP-ACP concentration may be attributed to the increased filler content leading to a lower monomer density, and the increased viscosity hindering adequate infiltration of the adhesive into the collagen network of dentin, thereby compromising bonding performance [34, 35]. Importantly, this reduction may be clinically relevant, as insufficient resin infiltration can impair hybrid layer formation and potentially reduce the long-term durability of the adhesive interface. Therefore, although the incorporation of higher concentrations of CPP-ACP may provide benefits in terms of enamel bonding, its negative impact on dentin bonding should not be overlooked.Nevertheless, according to previous studies, dentin bonding

agents are expected to achieve shear bond strength values in the range of 15–20 MPa [36]. In the present study, the mean dentin bond strength observed in the 3% CPP-ACP group was 16.66 MPa, which falls within the clinically acceptable range reported for dentin. However, although this value remains within this range, it represents a decrease compared to lower concentrations, which may indicate a potential reduction in bonding efficiency. Therefore, while the 3% CPP-ACP concentration can still be considered clinically acceptable in terms of bond strength, the potential adverse effect of increasing CPP-ACP content on dentin bonding performance should be taken into account. On the other hand, enamel bonding was not adversely affected by increased CPP-ACP concentration, which may be related to the compact nature of enamel, its high hydroxyapatite content, and the absence of a collagen matrix, features that distinguish it from dentin and make it less sensitive to changes in adhesive viscosity and infiltration dynamics.

Al Qahtani et al. have reported that fracture mode classification provides valuable insights for interpreting the results of shear bond strength tests [37]. In our study, adhesive-type failures were predominantly observed in all samples, regardless of whether the bond strength was high or low, and no significant correlation was found between fracture type and bond strength. Several studies in the literature have also stated that adhesive failures serve as a reliable criterion for accurately assessing bond strength [38, 39].

The FE-SEM analysis of enamel bonding interfaces revealed that the addition of CPP-ACP resulted in a more continuous and homogeneous interface morphology compared to the control, although the differences between the two CPP-ACP concentrations (1% and 3%) were minor. Based on visual assessment of the images at the same magnification, these observations suggest that CPP-ACP incorporation may be associated with a more uniform enamel–adhesive interface, even if the improvement at 1% does not translate into a statistically significant increase in bond strength.Furthermore, The FE-SEM analysis of dentin bonding interfaces revealed that the 1% CPP-ACP group exhibited visually longer and denser resin tags compared to both the control and the 3% CPP-ACP group. This suggests that a low concentration of CPP-ACP may improve resin infiltration into the dentin by modifying the smear layer and enhancing the interaction with exposed collagen fibrils. ACP nanoparticles also infiltrate the collagen matrix and organize themselves into a crystalline phase to facilitate dentin remineralization [40]. It has been reported in the literature that CPP-ACP promotes crystal reorganization on the dentin surface through the release of calcium and phosphate ions, which could positively influence adhesive penetration [41]. This biomimetic remineralization process may contribute to the formation of a substrate more receptive for resin infiltration, potentially supporting hybrid layer formation and bond durability. Conversely, a higher CPP-ACP concentration may result in surface precipitation that partially occludes dentinal tubules, thus limiting resin penetration and reducing micromechanical retention. The observed trend of enhanced tag formation in the 1% group, based on visual assessment, may indicate improved hybrid layer integrity and could potentially support long-term bond durability.Contact angle and surface tension are key for adhesive wettability. In our study, all adhesives had contact angles below 90°, with no notable differences between groups. Accordingly, the fourth null hypothesis, "The incorporation of CPP-ACP into the adhesive resin does not alter the material's contact angle and surface tension", was accepted. This outcome may be attributed to the relatively low concentration of CPP-ACP incorporated into the adhesive resin.

Clinically, the results of this study suggest that incorporating CPP-ACP into adhesive resin systems may provide added benefits without compromising essential adhesive properties when used at appropriate concentrations. The observed antibacterial activity against *S. mutans*, together with acceptable polymerization and bond strength values, indicates a potential role for CPP-ACP-modified adhesives in supporting caries prevention at the tooth–restoration interface. In addition, the concentration-dependent effects on enamel and dentin bonding highlight the importance of optimizing CPP-ACP content to balance bioactivity and adhesion. Although long-term durability and remineralization were not directly assessed, the localized release of calcium and phosphate ions may contribute to maintaining interfacial stability under acidic and cariogenic challenges. Further aging and in-vivo studies are required to clarify the long-term clinical implications of these findings.

Although the present findings contribute to the understanding of CPP-ACP-modified adhesive systems, the in vitro nature of the study should be considered when interpreting the results. The experimental study was conducted using a single commercial universal adhesive, which was evaluated solely in self-etch mode, while direct remineralization, thermocycling, water aging, or long-term degradation assessments were not performed.Moreover, the antibacterial activity assessment was limited to selected bacterial species (*S. mutans* and *L. casei*) and evaluated using an agar diffusion method, which may not fully reflect the clinical behavior of resin-based materials. In addition, it should be noted that direct measurement of calcium and phosphate ion release was not performed in this study, which limits the ability to fully interpret the proposed mechanisms related to radical stabilization and remineralization. Inclusion of such analyses in future studies would provide more comprehensive insight into the biological and polymerization-related effects of CPP-ACP. Investigating the effects of CPP-ACP

across a broader spectrum of microorganisms would allow for a more comprehensive evaluation of its antibacterial potential and help to better elucidate its possible clinical benefits. Future studies incorporating aging protocols, alternative antibacterial assessment methods, and in vivo models are therefore warranted.

Conclusion

The results of our study highlight the potential benefits of CPP-ACP incorporated into adhesive resins in dental practice. Specifically, the incorporation of CPP-ACP into the tested universal adhesive resin affected bonding performance in a concentration- and substrate-dependent manner, with 1% CPP-ACP enhancing dentin bond strength and 3% CPP-ACP improving enamel bonding, while also resulting in increased inhibition zones against *S. mutans* under agar diffusion conditions and maintaining acceptable polymerization efficiency. These findings suggest that CPP-ACP may be a promising component for modifying adhesive materials in clinical applications, offering potential functional and antimicrobial benefits.

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Data availability Data available on request from the authors.

Declarations

Conflict of interest No potential conflict of interest was reported by the author(s).

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