

# Effect of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) on bond strength of a universal adhesive to demineralized dentin

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## Abstract

**Objective:** This study investigated the effect of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) on the shear bond strength (SBS) of a universal adhesive in self-etch mode to demineralized dentin.

**Methods:** Seventy-five dentin specimens were randomly divided into five groups (n=15) as follows: Group 1, intact dentin; Group 2, demineralized dentin receiving no remineralization agent; and Groups 3-5, demineralized dentin receiving CPP-ACP for 30, 90, and 180 seconds, respectively. All groups were bonded by Adper Single Bond Universal adhesive in self-etch mode. After 24 hours, the SBS test was conducted, and failure modes were recorded. The data were analyzed by one-way ANOVA, Tukey post-hoc test, and chi-square test at the significance level of  $P < 0.05$ .

**Results:** The highest and lowest mean SBS values were observed in groups 1 (intact dentin:  $29.58 \pm 8.13$  MPa) and 2 (demineralized dentin:  $13.41 \pm 4.85$  MPa), respectively. ANOVA revealed a significant difference in SBS among the groups ( $P < 0.001$ ). A pairwise comparison revealed that the SBS of demineralized groups (groups 2-5) was significantly lower than that of the intact samples ( $P < 0.05$ ). Group 5, with CPP-ACP, applied for 180 seconds, showed a significantly higher SBS ( $20.74 \pm 4.54$  MPa) compared to group 2 ( $P < 0.05$ ).

**Conclusions:** Applying CPP-ACP paste for three minutes can increase the bond strength of the universal adhesive in self-etch mode to demineralized dentin, whereas shorter application times are not effective.

**Keywords:** Bond strength, Casein phosphopeptide-amorphous calcium phosphate, Composite Resins, Dental adhesives, Self etch, Tooth demineralization

## Introduction

One of the primary goals of conservative dentistry is to preserve dental hard tissue and maintain tooth

vitality by retaining the affected dentin in deep cavities (1, 2). However, resin materials' adhesion to caries-affected dentin is considerably lower than that to sound dentin (3, 4). It should be noted that although caries-affected dentin has less mineral content, its collagen matrix remains intact and can be remineralized (5).

Surface pretreatment with remineralizing agents has been proposed to strengthen demineralized dentin and improve the bond strength of adhesive materials to caries-affected dentin. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is a milk protein derivative that deactivates enzymatic degradation processes and remineralizes enamel and dentin by releasing and stabilizing calcium and

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phosphate ions and promoting their incorporation into the hydroxyapatite crystals (6, 7). The application of CPP-ACP-containing pastes, commercially known as GC Tooth Mousse or MI Paste (GC Corporation, Tokyo, Japan), on demineralized dentin may improve bond strength and durability, particularly when self-etch adhesives are applied (7-11). The proposed mechanism of CPP-ACP action involves increasing the environment's pH and providing minerals that react with 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) monomers of self-etch adhesives (12, 13).

There is no consensus on the appropriate application time of CPP-ACP on demineralized dentin before the bonding process. A previous study demonstrated that applying MI Paste for three minutes enhanced the shear bond strength (SBS) of carious dentin lesions (14). Another study by Elmalawany et al. (7) found that pretreatment of demineralized dentin with MI Paste for five minutes and one month restored bond strength to levels similar to sound dentin. However, this procedure is time-consuming and either increases chair-side time or necessitates delivering a temporary restoration.

The latest generation of adhesive systems, "universal" or "multimode" adhesives, can be applied not only on dental tissues but also on various substrates, including resin composites, ceramics, zirconia, and metal alloys, with no need for additional primers. They can be utilized in the self-etch or etch-and-rinse mode in dental substrates. These adhesives typically contain silane and a functional monomer, such as 10-MDP, which promote resin adhesion to different substrates (15). Previous studies have shown that these adhesives produce favorable and durable bonding to sound and caries-affected dentin when applied in both modes (15-21).

Scotchbond™ Universal Adhesive, also named Single Bond Universal Adhesive (3M ESPE, St. Paul, Minnesota, USA), contains MDP functional monomer and polyalkenoate copolymer, which chemically bond to hydroxyapatite (22). This universal adhesive may produce better adhesion to demineralized or caries-affected dentin than conventional etch-and-rinse adhesives, although some controversies remain in the literature (16, 17, 23).

This in vitro study aimed to investigate the effect of different application times (30, 90, and 180 seconds) of CPP-ACP paste (MI Paste) on the SBS of a universal adhesive in self-etch mode to demineralized dentin. The null hypothesis was that none of the application times of MI Paste would affect the SBS of Single Bond Universal adhesive to artificially demineralized dentin.

## Materials and methods

### Study design

The protocol of this in vitro study was approved by the ethics committee of Mashhad University of Medical Sciences (IR.MUMS.DENTISTRY.REC.1397.024).

### Sample size calculation

The sample size was calculated using G Power software (version 3.1, Heinrich-Heine Dusseldorf University, Dusseldorf, Germany) with a 95% confidence interval and 80% power. According to previous data provided by Moosavi and Nemati-Karimooy (14), the sample size for each group of this study was calculated to be 15.

### Sample preparation

Seventy-five caries-free human third molars, which were extracted due to periodontal and orthodontic indications, were selected. After cleaning the samples, they were examined using a stereomicroscope (Dino lite Pro, Anmo Electronic, New Taipei City, Taiwan) under  $\times 10$  magnification to exclude those with caries, erosion, enamel cracks, deep grooves, or fractures. The selected teeth were kept in 0.1% thymol solution for one week and then placed in 0.9% saline at room temperature until the time of the experiment. All samples were tested within two months after extraction.

At first, the occlusal enamel of teeth was entirely removed to expose the dentin surfaces. The specimens were examined under a stereomicroscope (Dino lite Pro, Anmo Electronics Corp, Taiwan) with  $\times 10$  magnification to ascertain no enamel was left. The teeth were then embedded in self-cure acrylic resin (Acropars, Marlic Co., Tehran, Iran) using plastic tubes to obtain a flat dentin surface parallel to the horizon. Samples were polished using 400- to 1200-grit silicon carbide papers (Starcke; Germany) for 60 seconds under running water.

### Study groups

The samples were randomly divided into five groups ( $n=15$ ). Group 1 received no treatment and was considered the positive control group.

The specimens allocated to groups 2-5 were subjected to a pH-cycling procedure based on the study of Kucukylmaz et al. (24). The demineralizing solution contained 2.2 mM  $\text{NaH}_2\text{PO}_4$ , 2.2 mM  $\text{CaCl}_2$ , and 50 mM acetic acid with a pH of 4.8. The remineralization solution contained 0.9 mM  $\text{NaH}_2\text{PO}_4$ , 1.5 mM  $\text{CaCl}_2$ , and 0.15 mM KCl, with a pH of 7.0. Each specimen was immersed in 10 ml of demineralizing solution at room

**Table1.** Materials used in this study and their compositions and application procedures provided by the manufacturers

| Product               | Type                     | Manufacturer                      | Batch number | Chemical composition                                                                                                                                                                                                                                                                                                                              | Application procedures                                                                           |
|-----------------------|--------------------------|-----------------------------------|--------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| Single Bond Universal | Universal adhesive       | 3M ESPE, St. Paul, Minnesota, USA | 618806       | HEMA, BISGMA, 2-propenoic acid, 2-methyl-, reaction products with 1,10-decanediol and phosphorous oxide, ethanol, water, 2-propenoic acid, 2-methyl-, 3-propyl ester, reaction products with vitreous silica, copolymer of acrylic and itaconic acid, camphorquinone, dimethylaminobenzoate, ethyl methacrylate                                   | Apply for 20 s with light brushing motion, air thin for 5 s, Light cure for 10 s.                |
| Filtek Z250           | Universal Composite      | 3M ESPE, St. Paul, Minnesota, USA | N915750      | TEGDMA < 1–5%, Bis-GMA < 1–5%, Bis-EMA 5–10%, UDMA 5–10%, silica and zirconia filler (82 wt % / 60 vol%), silica cluster filler                                                                                                                                                                                                                   | Place up to 2 mm thickness on the surface and light cure for 40 s.                               |
| MI Paste              | CPP-ACP Containing Paste | GC Corporation, Tokyo, Japan      | 4830416      | Glycerol, 5%–10% casein phosphopeptide-amorphous calcium phosphate, pure water, zinc oxide, sodium carboxyl, methyl cellulose, xylitol, D-sorbitol, silicon dioxide, phosphoric acid, titanium dioxide, guar gum, sodium saccharin, ethyl-p-hydroxybenzoate, magnesium oxide, propylene glycol, butyl-p-hydroxybenzoate, propyl-p-hydroxybenzoate | Apply the paste on the surface, leave it undisturbed for 3 minutes, and clean, but do not rinse. |

temperature for 8 hours, followed by 16 hours of immersion in the remineralizing solution. The solutions were renewed daily, and the cycles were repeated for 14 days.

To confirm dentin demineralization, the microhardness values of three specimens of each demineralized group were tested under a 50-gram load for 10 seconds with a hardness tester (Micro Hardness Tester, Koopa Pazhoohesh, Iran, Model: MH3). Three measurements were taken for each sample, and the average value was noted as the surface hardness value. The microhardness values were between 30-40 Knoop hardness number (KNH), similar to hardness values reported for natural caries-affected dentin (25).

The specimens of groups 1 (positive control group) and 2 (negative control group) did not receive any additional treatment. For groups 3-5, a CPP-ACP-containing paste (GC Tooth Mousse, GC, Tokyo, Japan) was applied on the dentin surfaces for 30, 90, and 180 seconds, respectively. Then, the remineralizing agent was removed with a wet napkin.

The Single Bond Universal adhesive (3M ESPE, St. Paul, Minnesota, USA) was applied to the surface of the specimens in all groups for 20 seconds with a light brushing motion. The adhesive was then air-thinned for 5 seconds and light-cured for 10 seconds by Bluephase C8 (Ivoclar Vivadent, Schaan, Liechtenstein) light-curing device with an 800 mW/cm<sup>2</sup> intensity. Cylindrical molds with 1 mm diameter and 3 mm height were placed on the surface of the specimens, filled with Filtek Z250 resin composite (A2 dentin shade; 3M ESPE), and light cured for 40 seconds. Table 1 presents the materials used in this study, their chemical compositions, and their application procedures.

#### *Thermal aging process*

After bonding, all the specimens were immersed in distilled water and stored inside an incubator (Fine Tech, Shin Saeng, Gyeonggi-do, South Korea) at 37 °C and 100% humidity for 24 hours to simulate the oral cavity temperature and complete the polymerization process. The thermocycling procedure was conducted between 5

and 55°C for 1000 cycles, with a dwell time of 30 seconds (Nemo, Tehran, Iran).

### Bond strength and failure mode assessments

A universal testing machine (SANTAM, Tehran, Iran) with the crosshead speed set at 1 mm/min was used for shear bond strength measurement. After performing the SBS test, the samples were examined under a stereomicroscope (Dino lite Pro, Anmo Electronics Corp, Taiwan) with  $\times 30$  magnification. The failure modes were categorized as adhesive (failure between the dentin and resin composite), cohesive (failure in the resin composite or dentin), and mixed (a combination of adhesive and cohesive failures).

### Statistical Analysis

The SBS data were analyzed using SPSS statistical package version 22 (IBM Corp., Armonk, New York, USA). The Kolmogorov–Smirnov test confirmed the normal distribution of the data ( $P > 0.05$ ). SBS values among different groups were compared by one-way ANOVA and Tukey post hoc test for pairwise comparisons. The chi-square test was used to compare failure modes among the groups. The statistical significance was set at  $P < 0.05$ .

## Results

The SBS values of different study groups are presented in Table 2. According to ANOVA, there was a significant difference between the study groups regarding SBS ( $P < 0.001$ ). The Tukey post hoc analysis showed that SBS values of the demineralized dentin groups (groups 2-5) were significantly lower than that of group 1 (intact dentin:  $29.58 \pm 8.13$  MPa) ( $P < 0.05$ ; Table 2). Furthermore, the mean SBS of Group 5, in which GC Tooth Mousse was applied for 3 minutes ( $20.74 \pm 4.54$

MPa), was significantly higher than that of Group 2 ( $13.41 \pm 4.85$  MPa) ( $P < 0.05$ ). The SBS values of the groups in which CPP-ACP was applied for 30 seconds (Group 3:  $17.28 \pm 7.76$  MPa) or 90 (Group 4:  $16.87 \pm 7.45$  MPa) seconds were comparable to each other and did not significantly differ from either group 2 or group 5 ( $P > 0.05$ ; Table 2).

Table 3 presents the frequency and percentage of failure modes after SBS testing for each experimental group. The adhesive failure was recorded as the most common failure mode in all study groups. According to the chi-square test, there was no statistically significant difference in the failure mode distribution between the study groups ( $P = 0.106$ ; Table 3).

## Discussion

The present study evaluated the SBS of a universal adhesive to demineralized dentin after treatment with different application periods of CPP-ACP. The adhesive used in this study was Single Bond Universal, which was used in the self-etch mode. Single Bond Universal or Scotchbond™ Universal are the same adhesives with different product names sold in different regions worldwide.

In the present study, the SBS of untreated demineralized dentin ( $13.41 \pm 4.85$  MPa) was significantly lower than that of intact dentin ( $29.58 \pm 8.13$  MPa). This finding agreed with Hass et al. (18), who indicated reduced micro tensile bond strength ( $\mu$ TBS) values in caries-affected dentin compared to sound dentin. In contrast, some studies reported that using Single Bond Universal in etch-and-rinse and self-etch modes produced comparable bond strength in sound and artificially induced caries-affected primary and permanent dentin (17, 19).

**Table 2.** The mean, standard deviation (SD), minimum (Min), and maximum (Max) values of shear bond strength (MPa) in the study groups

| Study groups                            | Mean $\pm$ SD*                 | Min     | Max   |
|-----------------------------------------|--------------------------------|---------|-------|
| 1 (Positive control)                    | $29.58 \pm 8.13$ <sup>a*</sup> | 15.25   | 47.32 |
| 2 (Negative control)                    | $13.41 \pm 4.85$ <sup>c</sup>  | 5.00    | 19.24 |
| 3 (CPP-ACP application for 30 seconds)  | $17.28 \pm 7.76$ <sup>bc</sup> | 8.04    | 32.92 |
| 4 (CPP-ACP application for 90 seconds)  | $16.87 \pm 7.45$ <sup>bc</sup> | 8.81    | 33.06 |
| 5 (CPP-ACP application for 180 seconds) | $20.74 \pm 4.54$ <sup>b</sup>  | 14.9    | 30.40 |
| P-value                                 |                                | < 0.001 |       |

\*The groups with different superscript lowercase letters indicate a statistically significant difference at  $P < 0.05$ .

**Table 3.** The frequency and percentage (%) of failure modes in the study groups

| Study groups                            | Cohesive   | Adhesive    | Mixed      |
|-----------------------------------------|------------|-------------|------------|
| 1 (Positive control)                    | 2 (13.3 %) | 8 (53.3 %)  | 5 (33.3 %) |
| 2 (Negative control)                    | 0          | 15 (100 %)  | 0          |
| 3 (CPP-ACP application for 30 seconds)  | 0          | 13 (86.6 %) | 2 (13.3 %) |
| 4 (CPP-ACP application for 90 seconds)  | 0          | 13 (86.6 %) | 2 (13.3 %) |
| 5 (CPP-ACP application for 120 seconds) | 1 (6.6 %)  | 11 (73.3 %) | 3 (20 %)   |
| P-value                                 |            | 0.106       |            |

In this study, the application of CPP-ACP for 180 seconds (3 minutes) caused a significant increase in the bond strength of Adper Single Bond Universal in the self-etch mode to caries-affected dentin. However, lower application periods (30 and 90 seconds) did not positively affect adhesion. It is believed that calcium and phosphate ions of CPP-ACP can easily infiltrate into the porous lesion of demineralized dentin, deposit in the partially demineralized crystals, repair hydroxyapatite crystals, and promote dentin remineralization (7). The findings of this study, however, imply that an adequate application period is required to achieve these effects. Other studies applied CPP-ACP on demineralized dentin for 3 minutes (14), 5 minutes (7), 15 minutes (26), and one month (7) and observed significantly enhanced bond strength of self-etch adhesives to demineralized dentin.

Consistent with the results of this study, several studies indicated that utilizing CPP-ACP before applying a self-etch adhesive system could increase the SBS of resin composite to carious dentin (7, 13, 27). In contrast, some investigations could not determine the positive effect of CPP-ACP on the adhesion of universal adhesives to demineralized and sound dentin (8, 26, 28-30). Krithi et al. (28) found that the self-etch system yielded acceptable bond strength to demineralized dentin treated with NaF and NovaMin-containing dentifrices. However, the CPP-ACP-containing dentifrice did not improve dentin adhesion (28). The different results obtained in these studies may be related to the variations in adhesive brands, demineralization protocols, and the method of applying CPP-ACP paste. In the study of Krithi et al. (28), a slurry of MI Paste covered the tooth surface, brushed with an electric toothbrush

for 2 minutes, and the samples underwent remineralizing and demineralizing cycles.

In this study, the three-minute application of the remineralizing paste enhanced adhesion to demineralized dentin. However, it could not reach the same adhesion achieved in sound dentin. In contrast to the outcomes of this study, Elmalawany et al. (7) displayed that using CPP-ACP for five minutes and one month on demineralized dentin resulted in a micro tensile bond strength comparable to sound dentin. The higher dentin bond strength in the study of Elmalawany et al. (7) may be related to the longer application time of MI Paste and the type of adhesive used. They employed All Bond Universal, an ultra-mild universal adhesive (pH>3) in the etch-and-rinse mode.

It is believed that the adhesive type and the mode of application greatly impact its adhesion to dentin (7, 31). In the present study, CPP-ACP increased the bond strength of the universal adhesive in the self-etch mode to demineralized dentin. However, the same results may not be obtained in the etch-and-rinse mode. Some studies proposed that applying CPP-ACP before the bonding procedure might compromise the bond strength of etch-and-rinse adhesives (27, 31, 32). An etch-and-rinse adhesive requires a prior phosphoric acid application to remove the hydroxyapatite and prepare denuded collagen fibers for resin infiltration. The application of CPP-ACP may compromise the performance of the etchant because of dentin tubule obliteration, thus preventing resin penetration (30, 33-35). The effect of CPP-ACP on the bond strength of self-etch adhesives is different (8), as these adhesive systems cause partial dental tissue demineralization. Therefore, residual dentin minerals and preserved hydroxyapatite crystals around collagen fibers may improve bonding

quality, especially in the presence of functional monomers in adhesives. Yoshida et al. (9) revealed the interaction between MDP monomers in universal self-etch adhesives and calcium and phosphate deposited on dentin following the application of CPP-ACP, which created stable calcium-monomer salt and increased dentin mechanical properties and bond strength. It has been shown that mild self-etch adhesives with higher pH show greater bond strength to dentin (29). CPP-ACP can increase environmental pH with calcium phosphate and enhance the bond strength of mild self-etch adhesives. The pH of the Adper Single Bond Universal adhesive used in this study was approximately 2.7, and it is considered a mild self-etch adhesive.

The type of failure mode can describe, to some extent, the bonding quality between two substrates. In the present study, adhesive failure was the most frequent failure mode. The occurrence of more adhesive and less mixed or cohesive failures can indicate lower bond strength in the study groups. There was no significant difference in the distribution of failure modes among the groups. However, the highest percentage of mixed and cohesive failures was observed in the positive control group (sound dentin), which also showed the highest SBS value. This partially corroborates the relationship between failure mode and bond strength.

One of the limitations of this study was using only one adhesive system. Another limitation was the artificial process of dentin caries formation in a laboratory setting. Dental caries result from complex demineralization and remineralization cycles, which are affected by host-related factors such as nutritional habits, oral hygiene, saliva, and the use of remineralizing agents. Therefore, extrapolating the results of in vitro studies with caries substrates to clinical setting can be challenging. Some studies indicated that 3-4 months of GC Tooth Mousse application is required to cause a remineralizing effect on exposed collagen fibers and produce a more durable bond (7, 36), which is longer than that used in this study. Further clinical studies are suggested to assess the effect of various application durations of CPP-ACP on caries-affected dentin before applying different adhesive systems.

## Conclusions

Within the limitations of this in vitro study, applying CPP-ACP-containing paste (GC Tooth Mousse) for 180 seconds could increase the SBS of the universal adhesive in self-etch mode to demineralized dentin. The lower application periods of CPP-ACP did not yield a positive effect on adhesion. The bond strength of all

demineralized groups was significantly lower than that of sound dentin.

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## Conflict of interest

The authors declare no conflict of interest.

## Authors' contributions

AN designed and supervised the project. RHS prepared the samples and collected the data. MK and AS helped in data collection, statistical analysis, and interpretation. HSM helped in data collection and wrote and revised the original draft. All the authors read and approved the final manuscript.

## Ethical approval

The protocol of this in vitro study was approved by the ethics committee of Mashhad University of Medical Sciences (IR.MUMS.DENTISTRY.REC.1397.024)

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