



## Effect of Casein Phosphopeptide Amorphous Calcium Phosphate Conditioning on Microtensile Bond Strength of Three Adhesive Systems to Deep Dentin

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### ABSTRACT

**Objectives:** This study aimed to assess the effect of casein phosphopeptide amorphous calcium phosphate (CPP-ACP) on microtensile bond strength of three adhesive systems to deep dentin.

**Materials and Methods:** In this in vitro study, the occlusal surface of 30 sound human third molars was sectioned at 2 mm below and above the cemento-enamel junction. The samples were randomly divided into three groups of OptiBond Solo Plus, G-Bond, and Clearfil SE Bond. Before composite resin bonding, each group was divided into two subgroups with and without dentin preparation with CPP-ACP. The teeth were then sectioned into 1 mm rods (n=15), and their microtensile bond strength was measured at a crosshead speed of 0.5 mm/min. Data were analyzed using two-way ANOVA and post-hoc Bonferroni test.

**Results:** There was no significant difference in bond strength of OptiBond Solo Plus (P=0.44) and Clearfil SE Bond (P=0.67) with/without CPP-ACP. A significant difference was found between the two subgroups of G-Bond in this respect (P<0.001). The differences in microtensile bond strength among all subgroups were significant (P<0.05).

**Conclusion:** The microtensile bond strength in use of G-Bond is significantly higher following dentin treatment with CPP-ACP compared with no use of CPP-ACP.

**Keywords:** Casein Phosphopeptide-Amorphous Calcium Phosphate Nanocomplex; Clearfil Bonding Agent; Dentin; Tooth Remineralization

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### INTRODUCTION

Use of tooth-colored restorations has greatly increased due to esthetic demands of patients and advances in dentin bonding systems. Application of dentin bonding agents has improved conservative preparation of teeth [1]. Methacrylate adhesives are available in etch and rinse (three-step and two-step) and self-etch (one-step and two-step) forms [2].

Considering the increasing use of tooth-colored restorations, knowledge about the bond strength of these systems and influential factors in this respect seems necessary. Currently, acceptable bond strength of composite resin restorations is the major goal of manufacturers of dental materials [3].

Dentin is a biological structure and its characteristics change with its location

(superficial or deep), age of patient, and diseases. Thus, achieving a durable and strong bond to dentin is difficult [4]. Bond strength of tooth-colored restorations is mainly based on micromechanical retention. Resin tags penetrate deep into dentinal tubules and adapt to demineralized inter-tubular dentin and denuded collagen fibers [5]. Several studies have reported reduction in bond strength of agents to deep dentin [6,7]. Low bond strength of deep dentin compared with superficial dentin is due to higher number of tubules and their larger diameter, resulting in reduction in intertubular dentin, which serves as a scaffold for resin penetration [4]. Presence of higher organic content compared with minerals in deep dentin decreases monomer penetration [7, 8]. Reliable dentin bonding agents are necessary for long-term clinical success of composite resin restorations. It is believed that adhesives enhance the marginal seal and prevent secondary caries [9]. Low bond strength can cause early microleakage around the restoration margins and subsequent marginal discoloration, hypersensitivity, secondary caries, and pulpal irritation [10,11]. Efficacy of denting bonding agents for prevention of microleakage and marginal gap is still a matter of debate [12]. Many products have been suggested for caries prevention. Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) is among the commonly used products to slow down the progression of caries and enhance the remineralization of enamel lesions [13,14]. It is composed of two components.

The CPP component can transfer and stabilize calcium and phosphate. The ACP component releases calcium and phosphate ions to provide a super-saturated state. Calcium and phosphate ions are gradually released, deposited in partially demineralized crystals and result in reconstruction of apatite crystals. This mechanism may be effective in improving the bond strength to deep dentin by increasing its mineral content [1,13]. CPP-ACP is available in the form of a paste (MI Paste /Tooth Mousse) and decreases the demineralization and enhances the remineralization of

dentin [13,14]. Tooth Mousse contains CPP-ACP and affects the bond to dentin. It may also affect the function of etch and rinse adhesive systems [15]. Search of the literature yielded studies on the effect of CPP-ACP on bond strength of different adhesive systems to affected dentin. Kamozaki et al. [16] concluded that the CPP-ACP-based pastes did not affect the microtensile bond strength of softened dentin. Also, Bahari et al. [1] showed that CPP-ACP had no significant effect on microtensile bond strength of adhesive systems to carious affected dentin. In a recent study, Agob et al. [17] revealed that CPP-ACP increased the micro-shear bond strength of resin-modified glass ionomer cement to caries-affected dentin. The behavior of the self-etch and etch and rinse bonding agents in deep dentin is variable [18]. Also, the bond strength of adhesive systems to deep intact dentin is of vital importance in different cases such as traumatized teeth. Since limited previous studies have evaluated the effect of CPP-ACP on affected dentin, and no study has been considered the effect of this agent on deeper intact dentin, this study aimed to assess the effect of CPP-ACP on microtensile bond strength of three different adhesive systems to deep dentin.

## MATERIALS AND METHODS

This in vitro study was conducted on 90 sound extracted human molars (ethical code: 1394-01-35-2114). Debris and soft tissue residues were removed using a universal scaler. All teeth were immersed in 1% chloramine T solution at 4°C for one month prior to the study [1]. The occlusal surfaces of the teeth were sectioned using diamond discs (D + Z, Berlin, Germany) under water coolant to remove the occlusal enamel and reach the central groove. Deep dentin was accessed by sectioning 2 mm below the dentinoenamel junction [19]. The tooth surface was standardized using a 600-grit silicon carbide paper (Phoenix Beta, Buehler, Germany) under water coolant. The teeth were randomly divided into three groups for the application of three bonding agents, and each group was divided into two subgroups according to use/no use of CPP-ACP.

A total of 90 samples were included in this study and randomly divided into six subgroups (n=15). Table 1 shows the classifications of the six subgroups. Bonding agents used in this study included: OptiBond Solo Plus (Kerr Corp., Orange, CA, USA) (etch and rinse), Clearfil SE Bond (Kuraray Medical Inc., Okayama, Japan) (self-etch two-step), and G-Bond (GC Corp., Tokyo, Japan) (one-step self-etch).

**Table 1.** Classification of study groups

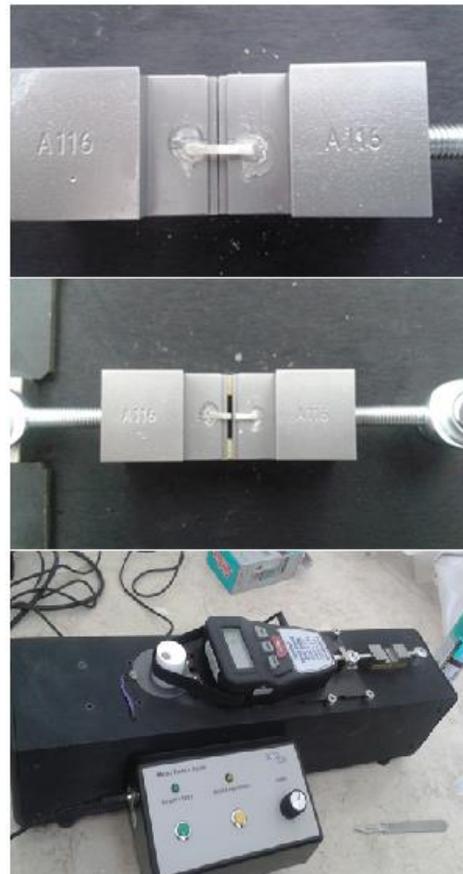
Groups	A	B	C	D	E	F
<b>Application of CPP-ACP</b>	-	+	-	+	-	+
<b>Bonding agent</b>	OptiBond Solo Plus	Clearfil SE Bond	G-Bond			

In all groups, the bonding agent was applied on the surface and light cured according to the manufacturer’s instructions. In groups B, D and F, before the bonding procedure, the CPP-ACP paste (GC Tooth Mousse, GC, Leuven) was applied on the tooth surface for five consecutive days each time for 15 min. It was then rinsed, and the bonding agent was applied similar to other groups. Composite resin (A2 G-aenial; GC corp., Tokyo, Japan) was directly applied on the bonded surfaces in three 1.5 mm increments to reach a final thickness of 4.5 mm. Each increment was cured for 20 s using a light curing unit with a light intensity of 600 mW/cm<sup>2</sup> (Dentamerica, CA, USA).

A Teflon-coated plastic instrument (composite placement instrument; Hu-Friedy, IL, USA) was used for placement of each composite increment. The samples were then incubated in distilled water at 37°C for 24 h.

The teeth were then glued to a plastic sheet using cyanoacrylate glue (Mad Wolf, Akpinar YapiMelemeleri, LTD, Istanbul, Turkey) and sectioned buccolingually and mesiodistally (Thin Sectioning Machine Inc., Rochester, NY, USA) under water coolant perpendicular to the bonding surface to obtain rods measuring 1 x 1 mm. Dimensions of the samples were measured at the bonding interface using a digital caliper (Mitutoyo, Tokyo, Japan).

After that, the samples were glued to the jig of a microtensile tester (Bisco, Schaumburg, USA) using cyanoacrylate glue (Akpinar YapiMelemeleri, LTD, Istanbul, Turkey) and subjected to tensile load at a crosshead speed of 0.5 mm/min until failure (Fig. 1).



**Fig. 1.** Samples were glued to the jig of a microtensile tester using cyanoacrylate glue and subjected to tensile load at a crosshead speed of 0.5 mm/min until failure

Fracture load in Newtons was converted to Megapascals (MPa) by dividing the load in Newtons by the surface area in square millimeters. The mode of failure was determined under a stereomicroscope (SMZ-800; Nikon, Osaka, Japan) at x40 magnification and reported in percentage for each group as follows: Type A: cohesive failure in dentin, type B: adhesive failure, type C: mixed failure (over 25% of fractures are type A) and type D: cohesive failure in composite resin.

**Table 2.** Microtensile bond strength and failure modes of the study groups

Groups	Microtensile bond strength				Failure modes Number (%)			
	Mean	Standard deviation	Minimum	Maximum	Cohesive in dentine	Adhesive	Mixed	Cohesive in composite
A	10.2	3.3	5.5	16	0	15 (100)	0	0
B	9.3	2.4	5.5	15	0	15 (100)	0	0
C	24.1	9.7	13	48	5 (3.33)	8 (5.33)	0	2 (1.33)
D	25.7	11.3	7.5	45.5	3 (20)	12 (80)	0	0
E	17.2	9.7	5.5	42	0	14 (9.33)	0	1 (6.66)
F	33.5	10.6	16.5	58	2 (1.33)	11 (7.33)	0	2 (1.33)

**Statistical analysis:**

Normal distribution of data was assessed using the Kolmogorov-Smirnov test ( $P > 0.05$ ). The mean and standard deviation of tensile bond strength were reported, and the effect of CPP-ACP and type of adhesive system on bond strength was analyzed using two-way ANOVA. The post-hoc Bonferroni test was used for adjustment of multiple comparisons. The equality of variances was assessed using the Levene's test. Data analysis was carried out using SPSS version 16 (SPSS Inc., IL, USA), and  $P < 0.05$  was considered significant.

**RESULTS**

Table 2 shows the microtensile bond strength in the six groups. The results of two-way ANOVA showed that type of adhesive system affected the mean microtensile bond strength ( $P < 0.001$ ). Also, the effect of surface treatment with CPP-ACP on microtensile bond strength was statistically significant ( $P = 0.002$ ). The interaction effect of type of adhesive system and surface treatment with CPP-ACP on microtensile bond strength was significant as well ( $P < 0.001$ ). Comparison of microtensile bond strength among the three adhesive systems based on the use and no use of CPP-ACP by the Bonferroni's test showed that the differences between groups A and C ( $P < 0.001$ ), B and D ( $P < 0.001$ ), D and F ( $P = 0.023$ ), and B and F ( $P < 0.001$ ) were significant. Also, the microtensile bond strength based on the application of CPP-ACP separately for the three adhesive systems was compared and the difference was only significant between groups E and F ( $P < 0.001$ ). The mode of failure in the six groups is shown in Table 2.

**DISCUSSION**

This study assessed the effect of CPP-ACP on microtensile bond strength of three adhesive systems to deep dentin and showed that both the type of adhesive and surface treatment with CPP-ACP affected the bond strength. The interaction effect of the two was also significant. Advances in bonding systems in the recent years have enhanced conservative tooth preparation [18]. The resin-dentin bonding mainly depends on micromechanical retention due to resin penetration between the exposed collagen fibrils of demineralized dentin [20]. Bonding to moist dentin such as affected dentin and deep dentin has always been challenging [21, 22]. Since the current adhesives are highly sensitive to excess moisture, bonding in these areas is challenging.

Also, monomer penetration decreases in deep dentin due to higher organic content compared with minerals [23-26]. CPP-ACP, a recently introduced product, has been shown to prevent demineralization and to increase remineralization by replacing both calcium and phosphate ions lost due to caries [27, 28]. Previous studies have reported promising results about the effect of CPP-ACP on bond strength of adhesive systems to affected dentin [13, 17, 29]. In this study, the most commonly used self-etch and etch and rinse bonding agents were chosen, and the effect of these adhesive systems on deep dentin was variable. Microtensile bond strength test is a valuable test for assessment of the efficiency of bonding [30]. However, it has some limitations. For example, samples need to be prepared in small size. The advantage of this test is that it enables equal load distribution compared with

the shear test. Thus, it decreases the frequency of cohesive failures in dentin [31]. The effects of CPP-ACP on sound and demineralized dentin have been evaluated in different studies. In accordance with this study, Shafiei et al. [32] stated that CPP-ACP pretreatment of exposed sound dentin could have a beneficial effect on bonding ability and interfacial integrity of self-adhering materials. Consistent with the results of the present study, Barbosa-Martins et al. [33] declared that by pretreatment with CPP-ACP, the microtensile bond strength of two different bonding systems to demineralized dentin could be enhanced. In addition, the influence of CPP-ACP and smear layer removal on resin-dentin microtensile bond strength was evaluated by Lin et al, [34] and they concluded that CPP-ACP application only increased the microtensile bond strength of G-bond (no use of EDTA) and Adper Single Bond 2 (using EDTA) after 3 days; but, after 6 months the impact of CPP-ACP was not significant. Although the results of Lin et al, [34] about the bond strength of G-Bond was similar to the present study, the time of bond strength assessment in this study was 1 day. Our study showed that the tensile bond strength to deep dentin depended on both the type of adhesive system and surface treatment with CPP-ACP. The results showed that surface treatment with CPP-ACP had no significant effect on the mean microtensile bond strength of OptiBond Solo Plus, which may be due to the use of phosphoric acid prior to the application of bonding agent, which decreases the concentration of calcium in the surface. Borges et al. [15] declared that phosphoric acid can completely denude the collagen fibers. It appears that it happens even in case of application of CPP-ACP. Thus, phosphoric acid can neutralize the efficacy of CPP-ACP in etch and rinse systems [15]. According to Yoshida et al, [35] the reaction between 10-MDP present in Clearfil SE Bond and superficial calcium can form calcium/monomer salt. Thus, it was expected that application of CPP-ACP on dentin surface increases the bond strength of Clearfil SE Bond to dentin by increasing the available calcium ions.

However, CPP-ACP had no significant effect on bond strength of samples in Clearfil SE Bond group. This finding was in accordance with this study and those of Borges et al, [15] and Sattabanasuk et al [36]. The possible explanation for this difference is the production of small amounts of calcium/monomer salt [15]. Also, it has been shown that following the application of CPP-ACP, protein components easily adhere to the tooth surface and provide a source of calcium and phosphate ions [14, 37]. High concentration of these ions on the tooth surface increases surface hardness due to mineral deposition [38]. Tooth surface following the application of CPP-ACP becomes more resistant to acid demineralization due to deposition of calcium and phosphate. The etching capacity of acidic conditioners especially weak acid primers in self-etch adhesive systems decreases in dental surfaces treated with CPP-ACP, which can subsequently decrease resin bond to tooth surface [39,40]. Application of CPP-ACP on dentin surface can decrease acid demineralization by one-third [14,37,41]. Scanning electron microscopic images have shown that even after rinsing the CPP-ACP paste, a thin layer remains on the tooth surface that can prevent resin penetration into the etched enamel and dentin and affect resin-tooth bond strength [39]. Moreover, it should be noted that lateral branches of tubules that connect intratubular resin tags decrease following the use of CPP-ACP, which may be due to mineralization and obstruction of channels [36]. These findings explain our results regarding no significant effect of CPP-ACP on bond strength of Clearfil SE Bond. However, further chemical analyses are required to confirm this hypothesis. Surface treatment with CPP-ACP may not have a positive effect on bond strength of Clearfil SE Bond but it does not have a negative effect on it either. Thus, this protocol may be considered for clinical use [29], since Yengopal and Mickenautsch [28], Oshiro et al, [14] Yamaguchi et al, [41] and Rahiotis and Vougiouklakis [37] showed that application of CPP-ACP had cariostatic effects and increased

remineralization and decreased demineralization of dentin. Also, Adebayo et al. [39] showed that CPP-ACP had no adverse effect on the morphology of dentin-adhesive interface and dentin bonding by self-etch two-step adhesives. Therefore, CPP-ACP can decrease susceptibility to secondary caries. Application of CPP-ACP had a significant effect on microtensile bond strength of G-Bond. The difference in the effect of CPP-ACP on Clearfil SE Bond and G-Bond may be related to the acidity of these two adhesives since Clearfil SE Bond has a pH of 2 and G-Bond has a pH of 1.5 [2]. Therefore, G-Bond primer with higher acidity can overcome the increased surface hardness after the application of CPP-ACP and penetrate into dentin and yield higher bond strength due to the availability of higher amounts of calcium for reaction with functional monomers. Fu et al. [42] indicated that samples treated with MI Paste prior to the application of Adper SE Plus (two-step self-etch adhesive) showed higher bond strength than untreated samples. Adper SE Plus has phosphoric acid esters that can chemically bond to hydroxyapatite. Thus, its bond strength can be improved by an increase in the available calcium ions following dentin preparation with MI Paste [42]. The higher bond strength of G-Bond may be explained by the fact that it also contains phosphoric acid esters; this statement was confirmed by reduction in the frequency of adhesive failures and increase in the frequency of cohesive failures after use of CPP-ACP in this group. Our study also compared the bond strength of the three adhesives with/without surface treatment with CPP-ACP. The results showed that in absence of CPP-ACP, the highest bond strength was noted in Clearfil SE Bond, followed by G-Bond and then OptiBond Solo Plus. These results may be due to the presence of functional monomers in these adhesive systems and their bond to dentin. Tsuchimoto et al, [43] and Iwai et al. [44] indicated that 10-MDP is the most efficient monomer in bonding to dentin. It is present in the composition of Clearfil SE Bond and participates in the bond to dentin by creating ionic chemical bonds with calcium ions present in dentin

crystallites. The second efficient monomer is 4-META, present in G-Bond followed by HEMA present in OptiBond Solo Plus [45]. Resin infiltration into the demineralized matrix must be as complete as possible to form a hybrid layer containing collagen fibrils reinforced with resin. However, scanning electron microscopic and transmission electron microscopic studies by Jacobsen and Söderholm [46], Milia et al, [47] Maciel et al, [48] Pashley and Carvalho [49] and Sano et al. [50] indicated that resin can less penetrate into the hybrid layer formed by the etch and rinse systems and it may be concluded that the collapse of collagen fibrils during the drying phase after rinsing of etchant is the main reason for deficient resin penetration. Their results can explain our findings regarding the lower bond strength of OptiBond Solo Plus compared with that of Clearfil SE Bond and G-Bond to dentin. According to Nakajima et al, [51] when Clearfil SE Bond primer is applied on normal dentin surface, it is partially demineralized to 1  $\mu$ m depth. Resin tags formed in this condition are narrow and cylindrical with some degrees of lateral penetration into the lateral branches of dentinal tubules, which seems to be sufficient for strong micromechanical retention during the bonding process. Sattabanasuk et al. [36] demonstrated that 10-MDP functional monomer which is present in the composition of Clearfil SE Bond can form a chemical bond with hydroxyapatite crystals remaining on the surface and increase the bond strength. The results of these studies are in agreement with ours regarding high bond strength of Clearfil SE Bond to dentin. Yoshida et al. [35] reported that 4-META in the composition of G-Bond can form ionic bonds to hydroxyapatite (similar to 10-MDP); however, the bond strength of 4-META is lower than that of 10-MDP and the resultant calcium salt is partially soluble and is not highly stable. This explains the lower bond strength of G-Bond to dentin compared with Clearfil SE Bond. In groups subjected to CPP-ACP, the highest bond strength was noted in G-Bond followed by Clearfil SE Bond and OptiBond Solo Plus. These results can also be explained by the afore-mentioned descriptions.

Application of CPP-ACP significantly increased the bond strength of G-Bond to deep dentin, and the highest bond strength was noted in this group. This study had an in vitro design and oral environment cannot be well simulated in vitro. Presence of saliva, occlusal loads, moisture, oral temperature, microbial plaque, and pulpal pressure can affect the results when applying dentin bonding agents to teeth in the oral cavity. Thus, future clinical studies are required to confirm the results of this study. Also, the bond strength of G-Bond to dentin following surface treatment with CPP-ACP must be evaluated in long-term.

### CONCLUSION

Within the limitations of this study, the results showed that surface treatment of dentin with CPP-ACP (Tooth Mousse) significantly increased its bond strength to G-Bond.

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### CONFLICT OF INTEREST STATEMENT

None declared.

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