Dental Research Journal

Original Article

The effect of remineralization pretreatments on the enamel bond strength of demineralized and sound enamel: An *in vitro* study

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ABSTRACT

Background: During tooth preparation, the clinician may face a hard remineralized enamel surrounding the cavity with unknown effects on the enamel bond strength. This study aims to assess the effect of remineralizing pretreatments with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) or CPP-amorphous calcium fluoride phosphate (CPP-ACFP) on the bond strength of composite resin and sound or demineralized enamel.

Materials and Methods: This study employed an *in vitro* experimental factorial design. A total of 144 enamel surfaces were prepared and randomly divided into 12 groups (G1-G12). The surfaces of G7-12 were demineralized to create a lesion (L), while G1-6 were assigned to the sound (S) enamel group. The three pretreatment protocols were CPP-ACFP, CPP-ACP, or no pretreatment for a 10-day pH-cycling period. A composite rod was bonded to the surfaces using a self-etch or total-etch bonding system. Shear force was applied, and the bond strengths of the specimens were measured. The data were analyzed using the Kruskal–Wallis test, followed by pairwise comparisons using Dunn's test. The significance level for all tests was set at 0.05.

Results: The sound (S) groups (33.81 ± 8.48) showed a significantly higher bond strength than the lesion (L) groups (25.77 ± 6.69). Among the pretreatments, CPP-ACFP-pretreated groups had the highest bond strength (33.86 ± 8.87). Pairwise comparisons showed significant differences between CPP-ACFP-treated demineralized enamel and control demineralized enamel in both bonding systems ($P = 0.019$ and $P = 0.025$ for Clearfil SE and Optibond FL, respectively).

Conclusion: Pretreatment of demineralized enamel with CPP-ACFP before using total-etch and self-etch systems results in a bond strength comparable to that of sound enamel, making it clinically acceptable.

Key Words: Composite resins, dentin-bonding agents, shear strength, tooth remineralization

Received: 27-Feb-2023
Revised: 30-Aug-2024
Accepted: 05-Oct-2024
Published: 24-Jan-2025

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INTRODUCTION

The shift towards minimally invasive dentistry emphasizes the importance of tooth structure preservation. This has promoted the use of remineralizing treatments and interventions in the early stages of caries development.[1]

Access this article online

Website: www.drj.ir
www.drjjournal.net
www.ncbi.nlm.nih.gov/pmc/journals/1480
DOI: 10.4103/drj.drj_118_23

How to cite this article: Shirani F, Malekipour MR, Azary M, Manesh VS, Gilani A, Mazdak A. The effect of remineralization pretreatments on the enamel bond strength of demineralized and sound enamel: An *in vitro* study. Dent Res J 2025;22:1.

Fluoride has long been used for caries prevention and treatment purposes.[2] Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and CPP-amorphous calcium fluoride phosphate (CPP-ACFP), which deliver bioavailable calcium and phosphate ions, have also been introduced as an adjunct to fluoride for non-invasive management of caries. It has been reported that CPP-ACP or its combination with fluoride has superior remineralizing effects compared to fluoride alone.[3] Further, CPP-ACFP can recover the tissue.

Several studies have investigated the impact of CPP-ACP and CPP-ACFP on the shear bond strength (SBS) of orthodontic brackets. However, these studies have yielded inconsistent results. Some studies have reported a significant decrease of SBS after application of CPP-ACP,[4,5] whereas others have observed a significant increase.[6] Additionally, some other studies have reported no significant difference.[7,8] The influence of CPP-ACFP on the SBS of orthodontic brackets has also been investigated in several studies. Among these studies, some have reported a statistically significant increase in SBS using CPP-ACFP,[7,9] while others have found no significant effect.[4,10]

Besides the impact on the bond strength of orthodontic brackets, the potential effects of CPP-ACP and CPP-ACFP are of significant concern in restorative dentistry. There are common clinical situations where a practitioner decides to adopt a conservative approach (e.g. a minimal composite resin restoration) and has to deal with the white spots. Leaving this carious enamel during preparation may jeopardize the success of restoration as it has been reported that a demineralized enamel has a lower SBS than a sound enamel.[11] It has also been suggested that the demineralized enamel should be eliminated prior to bonding.[12] Unlike demineralized enamel, the effects of remineralization of a previously demineralized enamel on SBS are unclear, and as discussed above, inconsistent findings have been reported. This inconsistency has raised questions, especially in high-risk patients who receive remineralization treatments and intermediate restorations as a means to counter the cariogenic activity of the biofilm. During the preparation of definitive restoration for these patients, clinicians often find themselves in a dilemma of either removing the remineralized enamel surrounding the cavity or leaving it as the aptness of this enamel to form a reliable peripheral seal is unclear.

Considering the inconsistent findings regarding the effects of CPP-ACP and CPP-ACFP on the SBS and the possible interactions of these remineralizing pretreatments with bonding type or mineral content of the enamel, this study was designed to determine the effects of bonding system, mineral content, and remineralizing pretreatment type on the enamel SBS.

The null hypothesis of this study was that there would be no difference in the SBS between the sound and demineralized enamel after applying the remineralizing agents and different bonding systems.

MATERIALS AND METHODS

This study utilized an *in vitro* experimental factorial design to investigate the effects of enamel condition (sound vs. demineralized), remineralizing pretreatments (CPP-ACFP, CPP-ACP, or no pretreatment), and bonding systems (self-etch vs. total-etch) on the SBS of composite resin to enamel.

Selection of teeth and preparation

This study was approved by the Regional Bioethics Committee of Isfahan University of Medical Sciences. One hundred and forty-four extracted human third molars without cracks or erosion, enamel hypoplasia, irregularities, and previous chemical treatment, which were extracted due to impaction during 2 months, were stored in 0.2% thymol solution. The enamel surfaces were polished with non-fluoridated pumice and a prophylactic cap. The teeth were washed with normal saline and were then dried. The specimens were mounted in self-curing acrylic resin (Simplex Rapid, Kemmdent, Associated Dental Products Ltd, Wiltshire, UK) cylinders so that the buccal surface of the crown was left outside the cylinder. The surface was coated with nail polish while a 5 mm × 7 mm window was left out on the buccal surface. The window surface was ground flat under water-cooling until a 4 mm × 4 mm flat area was visible.

Grouping the specimens

The teeth were divided into 12 groups (G1–G12). Six groups (G1–6) were allocated to the sound (S) enamel group and six groups (G7–12) were demineralized and included in the lesion (L) group to simulate the surface conditions of a white spot lesion. Three pretreatment protocols were considered before bonding the composite to the enamel surface:

A. Pretreatment with CPP-ACFP (MI Paste Plus, GC Corp, Tokyo, Japan) for G1, G2, G7, and G8
B. Pretreatment with CPP-ACP (Tooth Mousse, GC Corp, Tokyo, Japan) for G3, G4, G9, and G10
C. No pretreatment for the control groups (G5, G6, G11, and G12).

The odd-numbered groups (G1, G3, G5, G7, G9, and G11) were bonded with the self-etch bonding system (Clearfil SE Bond, Kuraray Medical Inc., Okayama, Japan), whereas the even-numbered groups (G2, G4, G6, G8, G10, and G12) were bonded with the total-etch bonding system (OptiBond FL, Kerr Italia S.r.l., Scafati, SA, Italy).

Demineralization process and pH cycling

The demineralizing and remineralizing solutions were made similar to those of Kumar *et al*.'s[13] experiments and were renewed each day for a constant pH throughout the test process. The lesion group (L) specimens were placed in the demineralizing solution (2.2 mM $CaCl_2$, 2.2 mM KH_2PO_4, and 0.05 M acetic acid, with its pH adjusted to 4.4 by adding 1 M KOH) to create subsurface enamel lesions. Similar to Abbas *et al*.'s study,[14] every specimen was examined with DIAGNOdent™ (KaVo, Bibberach, Germany) every day to determine the extent of the lesion. The specimens underwent this demineralization process until a reading of 10–25 (white spot lesions in the outer half of the enamel) was observed. The length of the demineralization process varied from 10 to 25 days. After this stage, both the lesion (L) and sound (S) groups underwent pH cycling for 10 days. All stages of the experiment were conducted in an incubator set to 37°C. Each daily cycle consisted of two demineralization phases and a remineralization phase. In each cycle, the specimens were first immersed in the demineralization solution (pH = 4.4) on a shaker for 3 h, followed by a 2-h remineralization phase (1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, 0.15 M KCl; pH = 7.0) and then a second demineralization phase for another 3 h. Pretreatments were carried out for each specimen before the first demineralization phase and both before and after the second demineralization phase. The paste was applied to the enamel and was then washed with normal saline solution 3 min later. After each day of pH cycling, the specimens were put in an artificial saliva solution (20 mmol/L $NaHCO_3$, 3 mmol/L NaH_2PO_4 and 1 mmol/L $CaCl_2$; neutral pH).[15]

Bonding procedure

Based on the grouping, one of the bonding agents was applied according to their manufacturers' instructions:

1. Two-step self-etch bonding system: The primer component was applied and rubbed on the surface using a microbrush for 20 s. The primer was air sprayed gently to evaporate the volatile ingredients. The bonding component was then applied using a microbrush, air-thinned to produce a uniform film, and light-cured (Demetron LC, Kerr, Orange, CA, USA) for 10 s
2. Three-step total-etch (etch and rinse) bonding system: The surface was etched using a 35% phosphoric acid gel (Ultra-Etch, Ultradent Products Inc., South Jordan, UT, USA) for 20 s, rinsed for 15 s, and gently air-dried to keep the surface moist based on a previous study.[16] The primer was applied using a microbrush, gently scrubbed on the surface for 15 s, and air-thinned afterwards. The adhesive was applied using a microbrush for 15 s and air-thinned for 3 s. Light-curing was performed on each surface for 20 s.

An acrylic tube with an internal hollow diameter of 2.5 mm and a length of 4 mm was placed on the prepared surface and filled with composite resin (Filtek Z250-A2 Shade, 3M ESPE Dental Products, St. Paul, MN, USA). Light-curing was performed from four sides around the tube for 40 s each time [Figure 1].

Storage and shear bond strength test

All specimens were kept in distilled water for 24 h and placed in an incubator at 37°C. They were subsequently tested in a universal testing machine (Dartec, Series HC10, England).

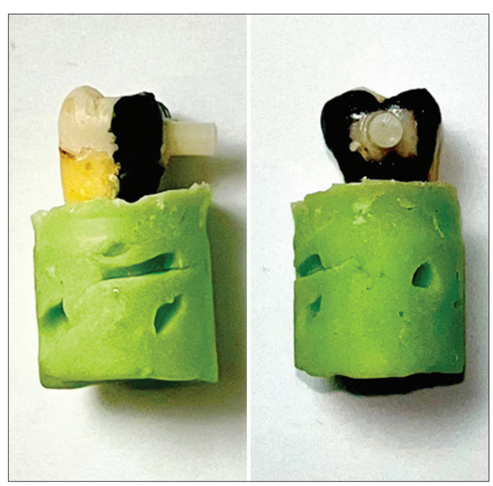

Figure 1: A prepared sample.

A knife-edge blade with a terminal thickness of 0.5 mm was fixed in the machine and a shear force perpendicular to the tooth was applied at a rate of 1 mm/min adjacent to the composite connection. The maximum load to failure (N) was recorded for each sample on the machine's monitor. The SBS (MPa) was calculated by dividing the load by the composite bonded surface. Each specimen was observed under a stereomicroscope at 40× magnification (Leica Ms5, Wetzlar, Germany) to determine the failure mode. Specimens with failure at the enamel-adhesive interface were classified as adhesive failure, whereas those showing any substrate parts were classified as cohesive failure.

Statistical tests

Due to the different bonding protocols for each bonding system and the difference in the appearance of specimens in demineralized, sound, and remineralized groups, blinding was not feasible for the researchers handling the specimens and treatments. Therefore, only the statistician and the technician responsible for performing the SBS test and recording the test results were blinded.

Statistical analysis was performed using IBM SPSS Statistical Software, version 26 (IBM Co., Armonk, NY, USA). The assumptions of normality and homogeneity of variances, required for three-way analysis of variance, were assessed. The Shapiro–Wilk test rejected the assumptions of normality for groups G9 and G11 (P = 0.008 and P = 0.002, respectively). In addition, the Levene's test indicated heterogeneity of variances (P = 0.023). Since these assumptions were violated, differences among the 12 experimental groups were compared using the non-parametric Kruskal–Wallis test, followed by pairwise comparisons between groups using Dunn's test.

The assumptions of normality and homogeneity were also tested for the main effects of lesion presence (sound/demineralized enamel), pretreatment (control/ CPP-ACP/CPP-ACFP), and bonding system (SE bond/Optibond Fl). The Shapiro–Wilk test rejected the normality for sound enamel (P = 0.005), demineralized enamel (P = 0.006), control (P = 0.015), CPP-ACP (P = 0.004), SE bond (P = 0.007), and Optibond Fl groups (P = 0.010). In addition, the Levene's test indicated the heterogeneity of variances for the bonding system (P = 0.023). Therefore, the nonparametric Mann–Whitney U-test was used to analyze the effects of lesion presence and bonding system, while the Kruskal–Wallis test was utilized to compare the three pretreatment groups.

To investigate differences in fracture patterns based on the variables, the Chi-square test was used. The significance level for all tests was set at 0.05.

RESULTS

The mean bond strength of the 12 experimental groups is presented in Table 1 and Figure 2. Among the studied groups, sound enamel treated with CPP-ACFP and OptiBond FL (G2) and demineralized control enamel bonded with Clearfil SE Bond (G11) had the highest SBS and lowest SBS, respectively. The Kruskal–Wallis test revealed a significant difference in the bond strength among the study groups (P < 0.001). Pairwise comparisons showed a significant difference between CPP-ACFP-treated demineralized enamel and the control demineralized enamel in both bonding systems (differences between G7 and G11; G8 and G12; P = 0.019 and 0.025, respectively). Regardless of the applied bonding system, there was no significant difference between the CPP-ACP-demineralized enamel and the demineralized control enamel (differences between G9 and G11; G10 and G12; P = 0.555 and 0.899, respectively).

As for the sound enamel groups, there was no significant difference between CPP-ACFP-treated groups and the control groups regardless of the applied bonding system (differences between G1 and G5; G2 and G6; P = 0.055 and 0.115, respectively). In addition, there was no significant difference between the CPP-ACP-treated sound enamel and the control sound enamel (differences between G3 and G5; G4 and G6; P = 0.291 and 0.879, respectively).

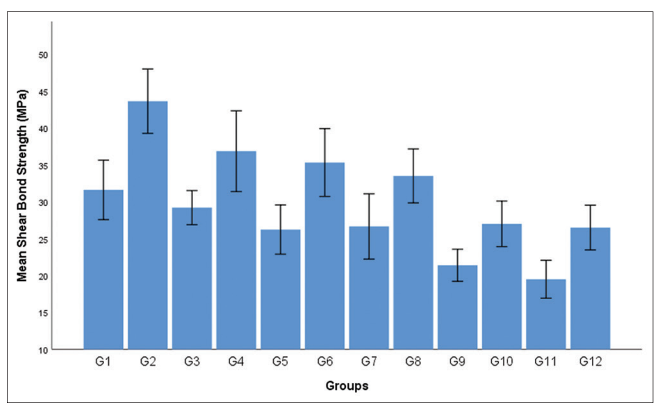

Figure 2: Mean shear bond strength in the experimental groups. The error bars represent 95% confidence Interval.

Table 1: Mean bond strength, MPa (mean±standard deviation) and 95% confidence interval (lower limit, upper limit) according to the experimental groups

	Bonding system	CPP-ACFP	CPP-ACP	Control
Sound	Clearfil SE Bond (Self-etch)	$31.62\pm6.35^{A,a,b,c}$ (27.59–35.65)	$29.21\pm3.65^{A,a,c}$ (26.89–31.52)	$26.24\pm5.25^{A,a}$ (22.90–29.57)
	OptiBond FL (Total etch)	$43.65\pm6.87^{A,}$d (39.28–48.01)	$36.87\pm8.61^{A,a}$ (31.40–43.34)	$35.33\pm7.2^{A,b}$ (30.73–39.92)
Lesion	Clearfil SE Bond (Self-etch)	$26.66\pm6.98^{A,a,c}$ (22.23–31.09)	$21.40\pm3.42^{A,b,b}$ (19.22–23.57)	$19.50\pm4.05^{B,c}$ (16.93–22.08)
	OptiBond FL (Total etch)	$33.52\pm5.76^{A,b,}$d (29.85–37.18)	$27.01\pm4.85^{B,b,c}$ (23.92–30.09)	$26.51\pm4.77^{B,a}$ (23.48–29.54)

Different lower-case superscripts within each column and different uppercase superscripts within each row indicate statistically significant differences ($P<0.05$). Results of the statistical analyses utilizing pair-wise comparisons with a significance level set at 0.05. CPP-ACFP: Casein phosphopeptide amorphous calcium fluoride phosphate; CPP-ACP: Casein phosphopeptide-amorphous calcium phosphate

Regardless of the bonding system, there was no significant difference between CPP-ACFP-treated demineralized enamel and control sound enamel (differences between G5 and G7; G6 and G8; $P = 0.885$ and 0.733, respectively). The mean bond strength was significantly higher in the sound (S) group (33.81 ± 8.48) than in the lesion (L) group (25.77 ± 6.69; $P < 0.001$).

The Kruskal–Wallis test showed a significant difference between the three pretreatments ($P < 0.001$). The *post hoc* Bonferroni corrected Dunn's test showed that CPP-ACFP-treated groups had the highest bond strengths (Mean = 33.86 ± 8.87) compared to the CPP-ACP (Mean = 28.62 ± 7.76; $P = 0.007$) and control groups (Mean = 26.89 ± 7.75; $P < 0.001$). There was no difference between the CPP-ACP-treated groups and the control group ($P = 1$).

There was a significant difference between the bonding systems ($P < 0.001$). The mean bond strengths of the self-etch and total-etch bonded specimens were 25.77 ± 6.50 and 33.81 ± 8.62, respectively.

The details regarding the failure patterns in each group are given in Table 2. The Chi-square test showed that the lesion presence caused a significant difference in the fracture pattern ($P < 0.001$). The effect of the bonding system on the fracture pattern was not statistically significant ($P = 0.051$). Overall, the 3 pretreatments did not show significantly different modes of failure ($P = 0.598$).

DISCUSSION

This study disproved the null hypothesis, demonstrating that both remineralizing agents and different bonding systems had prominent effects on the SBS.

The SBS of sound remineralized enamel was also assessed in this study since a portion of sound enamel would be affected during the remineralization treatments and this sound remineralized enamel might demonstrate a different behavior during the bonding processes in comparison with untreated sound enamel. For this purpose, intact impacted third molars were used as impacted third molars are less likely to be affected by external factors such as topical fluoride application and can provide a more homogeneous set of specimens. Unlike other teeth in the oral cavity, which may have been subjected to varying degrees of fluoride exposure due to routine oral hygiene practices.

The present study showed that the bond strength was higher in sound enamel than in demineralized enamel specimens (lesion groups). Porosities[17] due to mineral loss may jeopardize the bond strength and make it prone to cohesive failure as it was observed in this study. Suboptimal etching pattern has also been noted as a contributing factor.[11] This finding is in contrast with a previous report by Wiegand *et al*.[18] which demonstrated an overall higher SBS for specimens with demineralized enamel surfaces. However, this may be due to the use of caries infiltration systems for bonding purposes, which exhibit higher enamel penetration coefficients than conventional adhesives.[19] The groups in which a conventional adhesive system is used have been reported to have no statistically significant difference.[18] On the contrary, various studies[8,20-22] have observed that intact enamel shows a significantly higher SBS than demineralized enamel, which is consistent with the results of the present study.

Among the pretreatments, CPP-ACP did not compromise the bonding strength in sound enamel. Because of the prophylactic role of CPP-ACP in orthodontic patients, the effect of pretreatment on the bond strength of orthodontic brackets has also been tested. Similarly, Daneshkazemi *et al*. reported that the application of CPP-ACP to sound enamel does not compromise the bond strength of brackets using Transbond XT primer. Similar findings were reported

Table 2: Failure pattern in each group, enamel type, bonding agent, and treatment

	Adhesive, *n* (%)	Cohesive, *n* (%)	Mix, *n* (%)
G1	12 (100)	0	0
G2	9 (75)	3 (25)	0
G3	12 (100)	0	0
G4	12 (100)	0	0
G5	12 (100)	0	0
G6	10 (83.3)	0	2 (16.7)
G7	5 (41.7)	5 (41.7)	2 (16.7)
G8	5 (41.7)	4 (33.3)	3 (25)
G9	6 (50)	3 (25)	3 (25)
G10	6 (50)	4 (33.3)	2 (16.7)
G11	11 (91.7)	0	1 (8.3)
G12	4 (33.3)	8 (66.7)	0
Sound enamel	67 (93.1)	3 (4.2)	2 (2.8)
Demineralized enamel	37 (51.4)	24 (33.3)	11 (15.3)
Clearfil SE	58 (80.6)	8 (11.1)	6 (8.3)
OptiBond FL	46 (63.9)	19 (26.4)	7 (9.7)
Control (no treatment)	37 (77.1)	8 (16.7)	3 (6.3)
CPP-ACP	36 (75)	7 (14.6)	5 (10.4)
CPP-ACFP	31 (64.6)	12 (25)	5 (10.4)
Total	104 (72)	27 (18.8)	13 (9)

CPP-ACFP: Casein phosphopeptide amorphous calcium fluoride phosphate; CPP-ACP: Casein phosphopeptide-amorphous calcium phosphate

by Naseh *et al*. concerning the effects of CPP-ACP on the bond strength of brackets in sound enamel.[8,23] Gisoovar *et al*. investigated the effects of CPP-ACP on primary enamel after using one and two-step self-etch (AdheSE and AdheSE One F) and two-step etch and rinse (Tetric N Bond) systems, concluding that CPP-ACP does not influence the bond strength.[23] On the contrary, Shadman *et al*.[24] reported decreased SBS for AdheSE and AdheSE One F following the application of CPP-ACP. These conflicting results may be attributed to the technique sensitivity and different demineralizing/remineralizing protocols administered in each study.

The present study showed no significant difference between demineralized enamel treated with CPP-ACP and control demineralized enamel. In contrast, Akin *et al*. reported that the demineralized enamel increased the SBS compared to sound enamel after the application of CPP-ACP.[25] Daneshkazemi *et al*. also reported a higher SBS of brackets in the CPP-ACP-treated demineralized group, with no significant difference with sound enamel.[8] In another study conducted by Muntean *et al*., the SBS of CPP-ACP even surpassed that of the sound enamel.[7]

The results of sound enamel bond strength assessment showed that similar to CPP-ACP, CPP-ACFP did not jeopardize the bond strength of sound enamel when a total-etch or self-etch system was used. On the contrary, Al-Kawari and Al-Jobair[26] reported a significant decrease in the SBS of brackets following the application of CPP-ACFP before acid-etching. As it will be discussed later, the duration of acid etching may play an important role in the outcome of bonding after remineralization treatment and may explain this inconsistent result. These results may suggest that the operator can prescribe CPP-ACFP with no concern regarding the bond strength when the enamel is sound (e.g., bracket bonding).

In contrast with CPP-ACP, CPP-ACFP-treated demineralized enamel bonded with OptiBond FL showed a significantly higher SBS than control demineralized enamel to an extent comparable with the sound enamel. Ekizer *et al*. also tested the effect of pretreatments on the demineralized enamel. Orthodontic brackets bonded onto the surfaces pretreated with a mixture of CPP-ACP and 2% neutral fluoride were significantly stronger than the control group and the surfaces treated with 1.23% acidulated phosphate fluoride solution.[27] This has been attributed to the fluoride lowering the surface energy of the adherent and reducing its ability to spread.[27] However, the combination of CPP-ACP and fluoride increased the bond strength, which is in agreement with the results of the present study, showing that CPP-ACFP-pretreated groups had the highest bond strength.

Moreover, no significant difference was found between the enamel lesion treated with CPP-ACFP and sound enamel. Similarly, Uy *et al*.[28] reported no significant difference in the SBS between the CPP-ACFP-treated group and sound enamel. Cehreli *et al*. compared the effect of CPP-ACP and CPP-ACFP on the bond strength of brackets. They also reported a significantly lower bond strength in the CPP-ACP-treated group than in the CPP-ACFP group bonded with a total-etch adhesive system, which confirms the results of the present study. However, this difference was not observed between their self-etch bonded groups.[4] This may partly be due to their different pretreatment methods, as only a single remineralization treatment was carried out before the bonding procedure (compared to the 10-day pH cycling in the present study) and only on sound enamel. It has been speculated that the fluoride in CPP-ACFP does not decrease the bond strength because of its interaction with ACP, which makes both components ineffective and also deposits on the enamel as a nanocomplex, which does not drastically affect the bonding adhesives.[4]

The conflicting effects on the SBS after the application of remineralizing agents may be explained by their effects on the enamel structure. Remineralization treatment can strengthen the enamel and may therefore protect the bond from cohesive failure. It should be noted that this possible factor cannot explain the increased bond strength in the sound total-etch group since the majority of failures in the sound enamel are of adhesive type and other factors may also be involved. Soares *et al*.[29] reported a more prominent deposition of amorphous crystals or particles along peripheral prismatic enamel following the remineralization treatment. It can be concluded that remineralization increases the acid tolerance of the whole surface of enamel, but most of the acid tolerance lies in the peripheral prismatic enamel. Therefore, periphery prismatic enamel may show a greater acid tolerance than prismatic core during acid etching. This leads to a more pronounced difference in the dissolution of core and peripheral prismatic enamel and a more abundant type 1 acid-etch pattern (preferential prism core etching). In other words, the remineralizing agents intensify the selective dissolution of the prismatic core. Another outcome of hydroxyapatite deposition before the application of the SE system is the higher amount of calcium and phosphate to interact with the functional monomer of the SE system, 10-MDP, as Fukegawa *et al*.[30] showed 10-MDP readily reacted with phosphate and calcium hydroxyapatite.

The effects of remineralizing agents on the bond strength can be summarized as follows: (1) improved strength and resistance against cohesive failure, (2) intensified selective dissolution of prismatic core, (3) general resistance against acid-etching, which is a demoting factor to the bond strength, and (4) more interaction between 10-MDP and calcium and phosphate in the SE system. It can be assumed that any factor that diminishes the extent of acid-etching, including the insufficient acid-etching time, may turn this equation in favor of decreasing the bond strength. It has been reported[31] that acid-etching for 15s or less cannot produce the first acid-etch pattern and only roughens the enamel surface. This may explain the contradicting results of previous studies[24,32] that have applied 37% phosphoric acid for only 15s in contrast with the 20s acid-etching used in the present study. After remineralization treatment, a 15s acid-etch time may not be sufficient to overcome the increased acid tolerance of the enamel, which leads to a lower degree of enamel roughness. The total-etch bonding system showed higher bond strengths than the self-etch bonded groups. Previous studies have also shown similar results.[32]

The failure mode patterns indicate that most of the failures were of the adhesive type. Cohesive failures were more frequent in the lesion group than in the sound enamel group. This is because of the weakened structure of the demineralized enamel tissue after the formation of a defect. Failure patterns also showed no statistically significant difference among the treatments applied, which further supports the idea that CPP-ACP does not restore all of the lost mineral content of the lesion to the level of sound enamel.[33] Although the failure patterns seen in the bonding groups were not significantly different, it can be assumed that the results would be significant with a slightly higher sample size considering its low P value.

The observed superiority of bond strength in CPP-ACFP-treated enamel lesion over control enamel lesion is clinically significant. This implies that there is no need to extend the preparation to include the white spot to eliminate the low bond strength of the demineralized enamel, which promotes more conservative preparations. This study also showed that remineralizing treatments might be conducted before any procedure without concerns about the bond strength. The capacities of remineralization before bonding may not be restricted to carious enamel and may also be a promising way to remineralize and restore the hypo-calcified enamel in patients with Amelogenesis imperfecta whose conditions make dental adhesion challenging. This approach requires further studies.

The main limitation of this study is the inherent inaccuracy of *in vitro* studies in predicting clinical performance. It should be noted that unlike the settings of this *in vitro* study, the clinician often deals with enamel prisms parallel to the enamel/composite interface. It has been reported that the bond strength of enamel is weaker when the enamel/composite interface aligns with the orientation of enamel prisms as the weaker interprismatic enamel makes it prone to cohesive failure.[34,35] Therefore, it is safe to assume that the effects of demineralization or remineralization on the interprismatic enamel may also impact the bond strength by means yet to be studied.

Importantly, the present study did not consider the non-uniform degree of remineralization or demineralization in the enamel. Different parts of the enamel experience varying levels of these treatments, meaning that superficial enamel abundantly exposed to remineralizing agents may behave differently from the enamel adjacent to the dentinoenamel junction. This factor was not considered in this study as the SBS assessment was done using a ground flat enamel surface.

Another important limitation regarding the translation of the results to real clinical settings is the short duration of this study. Patients typically utilize remineralizing agents for extended periods, which may also impact the effects of the pretreatments on bond strength. This factor, however, requires further investigations.

It should be noted that the bond strength is not the only important factor involved in the success of the adhesive restoration. It has been shown that thermocycling as a simulator of an *in vivo* environment negatively affects the bond strength.[36] Therefore, it is suggested that a similar study be conducted using thermocycling to determine the bond strength and durability of remineralized enamel lesions in the dynamic environment of the oral cavity.

CONCLUSION

Within the limitations of the current study, it can be concluded that remineralizing agents not only do not compromise bond strength but can also increase the bond strength of enamel in the case of CPP-ACFP to the extent that remineralized enamel lesions do not significantly differ from sound enamel. In addition, using total-etch bonding systems such as OptiBond FL can result in higher bond strengths compared to Clearfil SE Bond.

Financial support and sponsorship
Nil.

Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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