

Shear Bond Strength of Composite to Primary Enamel Treated with Casein Phosphopeptide Amorphous Calcium Phosphate Using Total-Etch and Self-Etch Bonding Systems

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Abstract

Background and Aim: By application of casein phosphopeptide amorphous calcium phosphate (CPP-ACP) as a remineralizing agent, it may be feasible to only remove the carious dentin and better preserve the tooth structure. This study aimed to assess the shear bond strength of composite to primary enamel treated with CPP-ACP using total-etch and self-etch bonding systems.

Materials and Methods: This in vitro experimental study was conducted on 96 sound primary teeth randomly divided into 8 groups (n=12). Four groups were demineralized and the rest remained sound. All the specimens were subjected to pH cycling. Then, GC-Tooth Mousse was applied on their surfaces. Composite resin was bonded using Clearfil SE Bond self-etch or Single Bond total-etch bonding systems. The bond strength of the specimens was measured by an Instron machine and the mode of fracture was assessed by a stereomicroscope. Data were analyzed by one-way and two-way ANOVA and chi-square test.

Results: In the total-etch system, the bond strength of demineralized group was significantly higher than that of sound group (p=0.009). This difference in self-etch system was not significant (p=0.928). The CPP-ACP remineralizing agent decreased the bond strength in total-etch and increased the bond strength in self-etch group (p=0.032 and p=0.018, respectively). No difference was observed in the mode of fracture of the two bonding systems.

Conclusion: GC Tooth Mousse decreased the bond strength to composite in total-etch and increased it in self-etch group. Higher bond strength to composite can be achieved in teeth with white spot lesions (WSLs) when total-etch system is used.

Key Words: Primary teeth, Enamel, Remineralization, White spot lesion, Total-etch, Self-etch

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Introduction

Prevention of caries is much more important than treatment. Rampant caries and early childhood caries in young children are among the challenging issues in pediatric dentistry. Uncooperative children with these caries need to undergo general anesthesia for restoration of their teeth. Ceasing the

progression of carious lesions at early stages and their conservative restoration with tooth-colored restorative materials are among the main objectives of preventive programs in early childhood [1]. Since the administration of fluoride-containing compounds is limited and must be done with caution in children, treatment of these carious

lesions with other remineralizing agents available in the market is preferred and eliminates the need for unnecessary removal of tooth structure. The GC Tooth Mousse is among these agents available in dental markets [2]. It is a smooth, sugar-free, water-based mixture available for use as a topical crème with a nano-complex base of casein protein referred to as CPP-ACP. It is composed of CPP and ACP parts [3]. CPP localizes the ACP molecules on the tooth surface and bonds to biofilm macromolecules on the tooth surface and serves as a reservoir of calcium phosphate ions [4]. ACP is biologically active and capable of releasing calcium and phosphate ions to maintain a supersaturated level of these ions. Calcium phosphate ions in a liquid phase easily diffuse into the porous lesions and are deposited in the partially demineralized enamel crystals, reforming apatite crystals [5]. By doing so, the process of demineralization is slowed down and replaced by remineralization [5,6]. Positive efficacy of CPP-ACP for increasing the pH of the saliva has been shown in clinical studies [7]. The potentials of CPP-ACP [inhibition of demineralization, increasing remineralization, decreasing adhesion [8] and its bacteriostatic and bactericidal effects] are similar to those of fluoride, the gold standard cariostatic material [9-11]. Higher efficacy [4] and greater remineralizing potential of CPP-ACP compared to fluoride have been documented in several studies. CPP-ACP does not show any of the adverse effects of fluoride overdose such as fluorosis (moderate overdose) or toxicity (high overdose). The favorable efficacy of CPP-ACP in conjunction with fluoride has also been documented [13]. In an in-vitro study, CPP-ACP paste used following a fluoridated toothpaste in an erosive cycle resulted in less enamel loss compared to the single use of fluoride (50-250 ppm) or CPP-ACP. It was also shown that the acid resistance of enamel exposed to CPP-ACP was higher when fluoride was used in combination with CPP-ACP [14].

Considering the fact that in preventive strategies, remineralizing agents such as CPP-ACP are often used concomitantly with conservative restorations for initial caries, the effect of these materials on bond strength of composite restorations is an important topic in need of investigation. It has been shown that application of GC Tooth Mousse with and without bleaching does not prevent enamel etching; however, etching of enamel may

enhance the efficacy of self-etch adhesives after the use of GC Tooth Mousse [15]. Another study compared the bond strength of total-etch and self-etch adhesives after bleaching and treatment with CPP-ACP and showed that self-etch adhesives yielded the highest bond strength compared to other groups (bleached, treated with CPP-ACP and both) [16]. Bahari et al. evaluated the effect of this agent on carious dentin and showed that application of CPP-ACP had no negative effect on dentin bond to composite (irrespective of the type of bonding system used) [17].

Considering the fact that application of CPP-ACP on primary teeth is safe for children, this study aimed to assess the bond strength of composite to WSLs on primary teeth treated with CPP-ACP using total-etch and self-etch bonding systems.

Materials and Methods

In this in-vitro, experimental study, 96 human primary teeth extracted within the past three months with no cracks, abrasion, fracture, caries, restoration, fluorosis, decalcification or developmental defects in crowns were selected and stored in 0.2% thymol solution until the experiment. After removing the debris by a curette, the teeth were rinsed with saline and cleaned with a prophylactic cup and a hand piece. The specimens were mounted in auto-polymerizing acrylic resin to the level of the cemento-enamel junction. The teeth were randomly divided into eight groups of 12. Teeth crowns (out of the acrylic resin) were coated by nail varnish excluding a window measuring 5×5mm on the buccal or lingual surface. Each group was coated with a different color of nail varnish so that the groups could be distinguishable. Four groups were immersed in a demineralizing solution for three weeks to achieve enamel decalcification and the clinical chalky white appearance. Demineralizing agent was prepared based on a formula (pH of 4.4) [17]. After artificially creating WSLs in four groups, one non-demineralized and two demineralized groups were subjected to pH cycling. Specimens in each cycle were placed in a demineralizing solution twice daily each time for three hours on a shaker. Specimens were treated with GC Tooth Mousse (GC Corporation, Tokyo, Japan) before the first demineralization and before

and after the second cycle. The paste was applied on the enamel surface and cleaned with saline and a toothbrush after 15 minutes. Also, between the two demineralization cycles, the specimens were immersed in remineralizing solution on a shaker for two hours. After termination of the cycles in each day, specimens were immersed in artificial saliva. The remaining four groups (demineralized and non-demineralized) were stored in artificial saliva during the entire study period.

Ten days after the completion of treatment cycle, each specimen was evaluated under a stereomicroscope and it was noted that artificially induced WSLs had a change of color due to treatment with GC Tooth Mousse. Next, composite was bonded to the enamel surface in all control and intervention groups using total-etch (Single Bond, 3M ESPE Dental Products, St. Paul, MN, USA) or self-etch (Clearfil SE Bond, Kuraray Medical Inc., Okayama, Japan) bonding agents.

In the total etch system, the enamel surface was first dried and etched with 37% phosphoric acid for one minute, followed by rinsing with water for 15 seconds and air drying for 5 seconds. Adhesive was then rubbed on the surface by a microbrush for 15 seconds. Excess adhesive was thinned using mild air spray for 5 seconds. Another layer of adhesive was applied on the surface, thinned with mild air spray and light cured for 20 seconds (Demetron LC, Kerr, USA). In the self-etch system, the tooth surface was first dried, etched for 20 seconds, rinsed with water for 15 seconds and air dried for 5 seconds. Primer of the self-etch system was then rubbed on the surface by a microbrush for 20 seconds and excess primer was thinned by dry mild air spray. Bonding agent was then applied on the surface by another microbrush, thinned by gentle air spray and light cured for 10 seconds.

After applying the bonding agent on the enamel surface, plastic tubes with an internal diameter of 2.6mm and height of 5mm were filled with Valux™Plus composite resin (3M ESPE Dental Products, St. Paul, MN, USA) and placed on the treated enamel. Light curing was performed for 40 seconds from the top, 40 seconds from the right and 40 seconds from the left side.

In the next step, plastic cylinders were cut by a

scalpel and separated from the composite.

Specimens were immersed in distilled water in an incubator at 37°C for 24 hours. Bond strength of composite to enamel surface was tested in a universal testing machine (HC10, Dartec, UK). Specimens were fixed in the machine and shear load was applied by a blade with 0.5mm thickness vertically at a crosshead speed of 0.5 mm/minute to the nearest point to the composite-tooth interface. The load at fracture was displayed on the monitor in N. The load in N was divided by the surface area of the composite-tooth interface in mm² to obtain the shear bond strength value in megapascals (MPa).

Mode of failure was reported to be adhesive, cohesive or mixed. Adhesive failure was defined as complete separation of composite from the enamel. Separation of enamel from the tooth, or composite remaining on the tooth surface were referred to as cohesive, and a combination of cohesive and adhesive failures was defined as mixed failure. After failure, the surfaces were evaluated under a stereomicroscope to determine the mode of failure. Data were analyzed using Tukey's test and two-way ANOVA to assess the effect of two simultaneous variables. Chi square test was used to analyze the mode of failure.

Results

Table 1 shows the mean and standard deviation (SD) of bond strength in different groups. Two-way ANOVA showed a significant difference in bond strength in the total-etch bonding system between demineralized and sound (non-demineralized) groups ($p=0.009$). Bond strength in demineralized group increased. In other words, enamel lesion affected the bond strength but in the self-etch bonding system, the effect of demineralization was not significant ($p=0.928$).

In the total-etch and self-etch bonding systems, a significant difference existed in bond strength between treated and untreated groups ($p=0.032$ in total-etch and $p=0.018$ in self-etch systems).

In the total-etch system, the CPP-ACP decreased the bond strength while in the self-etch system, CPP-ACP increased the bond strength to composite. Two-way ANOVA showed that in both

Table 1. The mean and SD of bond strength in different groups based on the bonding system used

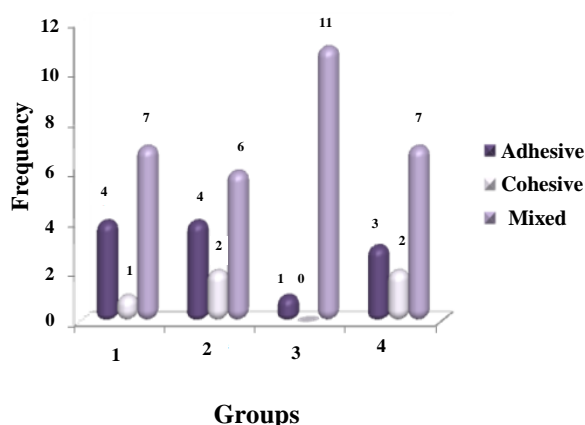
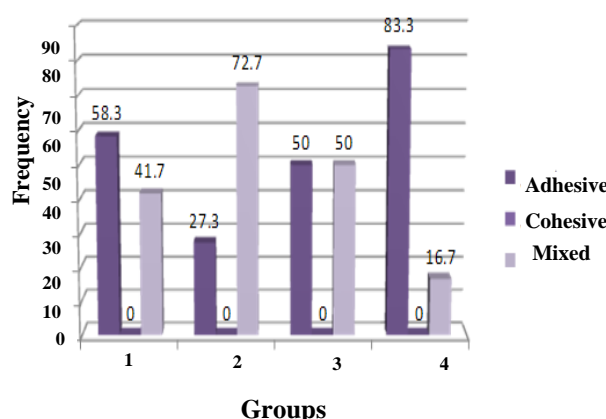
-	Bonding system	Treated	Untreated	Total
Demineralized	Clearfil SE Bond (self-etch)	27/97±8/46 ^{aA}	24/00±8/54 ^{bB}	25/89±8/53 ^b
	Single Bond (total-etch)	25/62±6/99 ^{bB}	30/38±4/84 ^{aA}	27/90±6/40 ^a
Non-demineralized (sound)	Clearfil SE Bond (self-etch)	29/82±8/17 ^{aA}	22/57±4/76 ^{bB}	26/19±7/51 ^b
	Single Bond (total-etch)	21/96 ±6/09 ^{bB}	24/76±5/07 ^{bA}	23/36±5/76 ^b

Lower-case letters indicate significant differences in columns and upper-case letters indicate significant differences in rows (p<0.05 was considered statistically significant)

self-etch and total-etch bonding systems, no interaction effect was found between the demineralizing and the therapeutic (CPP-ACP) agents. In other words, the effects of these two factors are independent of each other (p=0.473 in self-etch system).

In the total-etch system, one-way ANOVA showed a significant difference among groups (p=0.012). Post-hoc Tukey's test showed a significant difference between demineralized untreated and sound treated groups (p=0.007).

Chi square test was used to assess the mode of failure. In both bonding systems, chi square test showed no significant difference among the four groups with regard to the mode of failure (Diagrams 1 and 2)

**Diagram 1.** Frequency distribution of modes of failure in total-etch system**Diagram 2.** Frequency distribution of modes of failure in self-etch system

Discussion

The null hypothesis of the study was that application of CPP-ACP would have no effect on the bond strength of composite to sound and demineralized primary enamel using self-etch and total-etch bonding systems.

The results regarding the total-etch system showed that demineralizing agent increased the bond strength while a study by Shirani and Azari on permanent teeth showed no significant difference between demineralized groups treated with GC Tooth Mousse and the control group.

Demineralized treated group showed bond strength as high as that of sound enamel. A significant difference was noted in the mean bond strength of demineralized and non-demineralized (sound) groups, and bond strength in demineralized group was low. Also, Xiaojun et al. showed that CPP-ACP increased the bond strength of composite to enamel in permanent teeth [14]. This difference

between our results and those of studies on permanent teeth is probably due to differences of the primary and permanent enamel. Enamel is composed of 96%

minerals including hydroxyapatite crystals. Protein remnants left from the developmental phase and water are also found in the enamel [18]. Enamel has an intercellular matrix with 1-2 mm thickness in permanent teeth and 0.5-1mm thickness in primary teeth [19]. Morphological assessments showed that an aprismatic layer exists in the outermost enamel surface. Aprismatic layer is more commonly seen in primary teeth and this layer has a greater thickness in primary teeth. More than 60% of primary teeth have an aprismatic layer measuring 16-45µm in thickness while half the permanent teeth have an aprismatic layer with less than 5µm thickness [20].

In the self-etch bonding system, demineralization caused no significant difference in bond strength of composite to primary enamel among the study groups. In other words, bond strength of composite to sound and demineralized (with WSLs) primary enamel was not significantly different.

Demineralization may be slight or extensive (damaging the entire tooth structure). Thus, lack of a significant difference in bond strength of composite to sound and demineralized primary enamel does not necessary rule out the effect of demineralization; because in our study, demineralization was so slight that it was not visible when the teeth were moist and the chalky white appearance of WSLs was only visible when dry.

It seems that such a degree of demineralization does not have adequate porosity to affect the bond strength. However, greater degrees of demineralization can affect the enamel hardness and its bond to composite. Future studies on different degrees of enamel demineralization are required. The other null hypothesis of our study was that CPP-ACP would have no effect on the bond strength of composite to demineralized and sound primary enamel. Xiaojun et al. microscopically assessed and compared enamel treated with CPP-ACP and untreated enamel after etching and found that treated and etched enamel had a rougher surface than untreated enamel [14].

A rougher enamel surface provides a greater surface area for adhesive bonding, and higher number of resin tags ensures higher bond strength [21]. Moezzizadeh and Motamedi showed that application of CPP-ACP paste on dentin surface decreased the bond strength of light cure glass ionomer except when poly acrylic acid was used prior to the application of glass ionomer. These results are in accordance with our findings regarding the total-etch bonding system.

Application of polyacrylic acid conditioner on the dentin surface in specimens not exposed to CPP-ACP did not increase the bond strength [22].

Also, mode of failure was adhesive in all specimens and no significant difference was noted among groups. Similar results were obtained in the current study. In the total-etch system, treatment with CPP-ACP decreases the effect of bleaching and reduces the bond strength in primary teeth. However, in self-etch system, GC Tooth Mousse increased the bond strength of composite to primary enamel.

As described earlier, the mechanism of action of CPP-ACP is as follows: CPP bonds to calcium and phosphate via the phosphoserines in its structure and allows for the formation of small clusters of ACP, without allowing the clusters to reach their critical size for crystal growth and subsequent deposition of calcium phosphate. Thus, the normally insoluble calcium phosphate becomes soluble in presence of CPP [23-25].

In total-etch bonding system, application of etchant demineralizes the enamel by 100%. It can be assumed that application of an efficient remineralizing agent stabilizes the hydroxyapatites and interferes with the function of etchant.

On the other hand, in self-etch system, Clearfil SE Bond contains 10-MDP. This molecule can bond to calcium ions in hydroxyapatite crystals remaining around collagen due to partial demineralization. Self-etch system allows for this mechanism by partial demineralization of tooth structure.

Considering the role of CPP-ACP in forming hydroxyapatite crystals around collagen fibers and strengthening the bond, it is logical that CPP-ACP increases the bond strength when self-etch bonding system is used. Since no significant difference was noted in composite bond to demineralized treated,

sound treated and sound untreated groups, it can be concluded that application of CPP-ACP for remineralization of WSLs in primary teeth does not interfere with composite bond to sound and demineralized primary enamel.

In both bonding systems, mode of failure was not significantly different among groups. However, in demineralized untreated group, the most prevalent mode of failure was mixed, which indicates higher penetration of acid and resin into the enamel compared to other groups. In other words, demineralizing agent increased the effect of etching. Cohesive failures were more common in demineralized compared to non-demineralized (sound) group, which is due to the weak demineralized structure of enamel after the formation of WSL. Shirani and Azari in their study showed that cohesive failures were more common in demineralized compared to non-demineralized (sound) group, which was due to the weak demineralized structure of enamel after the formation of lesion. However, in the current study, demineralization was limited to formation of WSLs detectable only after drying the tooth surface. Obviously, further enamel demineralization undermines the bonding interface and may weaken the bond as in permanent teeth. Since no difference in bond strength of composite was noted to demineralized, treated and sound untreated primary enamel, further studies are required to further scrutinize and re-evaluate the necessity of removal of demineralized primary enamel at the margins during cavity preparation.

Conclusion

Based on the results, the following conclusions may be drawn:

1. Bond strength of composite to demineralized primary enamel treated with GC Tooth Mousse was acceptable and as high as that of sound enamel.
2. Application of GC Tooth Mousse decreased the bond strength of composite to primary enamel when total-etch bonding system was used. But, it increased the bond strength in self-etch system. Higher bond strength to composite can be achieved in teeth with WSLs when total-etch system is used. Self-etch bonding system did not cause a significant change in bond strength.

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