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Said Karabekiroğlu
Necmettin Erbakan University, Faculty of Dentistry, Department of Restorative Dentistry, Konya, Turkey

Işın Akdemir
Necmettin Erbakan University, Faculty of Dentistry, Department of Restorative Dentistry, Konya, Turkey

Fatma Betül Kahraman
Necmettin Erbakan University, Faculty of Dentistry, Department of Orthodontics, Konya, Turkey

Correspondence
Said Karabekiroğlu
Necmettin Erbakan University, Faculty of Dentistry, Department of Restorative Dentistry, Konya, Turkey

Influence of CPP-ACPF paste and fluorid varnish on bracket shear bond strength of two adhesive systems

Said Karabekiroğlu, Işın Akdemir and Fatma Betül Kahraman

Abstract
The aim of this *in vitro* study was to investigate the effect of CPP-ACPF paste and fluorid varnish (5% NaF) application before/after acid etching on the bracket-enamel shear bond strength (SBS) of a total etch system and to compare them one self-etch system. 160 teeth were divided into eight equal groups (20teeth/group), two control and six test groups: 1-Control etch, 2-CPP-ACPF before etch, 3-CPP-ACPF after etch, 4-%5NaF before etch, 5-%5NaF after etch; 6-Control-selfetch, 7-CPP-ACPF before self-etch, 8-%5NaF before self-etch. Debonding forces will be measured with a universal testing machine at a crosshead speed of 0.5 mm/min. Enamel surface treatment with %5NaF before acid-etching significantly decreased SBS compared to the control and to its application before acid-etching both etch and self-etch adhesives ($p<0.05$). Brackets SBS was not changed significantly when CPP-ACPF was applied after/before acid-etching. Significantly decreased SBS mean was obtained when %5NaF application was made before etching

Keywords: fluoride varnish, CPP-ACPF, enamel demineralization

1. Introduction

Incipient caries lesions (IL) around the surfaces of banded or bonded teeth are a common orthodontic problem, estimated to occur in 26% to 89% of patients. The presence of fixed orthodontic appliances further affects oral hygiene and makes the cleaning of teeth more difficult; hence, accumulation of plaque around brackets and bands increase, which causes enamel demineralization [1]. Although the patients are instructed about efficient oral care processes, early incipient caries lesions still remain as an actual clinical problem in fixed orthodontic appliances. These lesions cause problems such as unaesthetic and patient dissatisfaction after orthodontic treatment. These problems have made it important to determine patient's saliva, oral care condition and carries risks and evaluate, when necessary, to evaluate early-protective implementations before the orthodontic treatment. In order to handle this problem, treatment of enamel surface with various caries preventive materials has been suggested, before and during the orthodontic treatment [2-4].

In literature, there are available many studies working on solutions to prevent incipient caries lesions that occured during fixed orthodontic treatment [6, 7]. Mostly studies in the literature were focused on dental materials used for connecting to orthodontic bracket, or on sealing the buccal surfaces of teeth. Topical fluoride application by toothpastes, gels, rinses, and varnishes were found to be beneficial in patients with fixed appliances [6, 7]. It is a very important fact that the fluoride contributing the tooth to be more enduring by getting in the tooth enamel crystals is very effective in terms of caries prevention. Fluoride ions get into the structure of hydroxyapatite, and the soluble hydroxyapatite turns into a less soluble form such as fluorapatite and fluorhydroxyapatite [8]. In addition to this, it has been reported that fluoride applied enamel surface might affect the bracket bond strength and cause the bracket failure from the tooth surface [8]. Several methods of topical fluoride application in orthodontic practice are (1) before etching, (2) during etching when the fluoride is added to acid etchant, or (3) after etching (before bracket bonding) the enamel. However, the effects of different fluoride methods on bond strength of brackets have been previously reported with conflicting results [9].

Calcium and phosphate are major components of the teeth and concentration of these ions in the teeth is important. For this reason, in order to halt the incipient caries lesion and support the remineralization, it has been aimed to increase these ions in the tooth structure. Since casein phosphopeptide-amorphous calcium phosphate (CCP-ACP) stabilizes the necessary calcium and phosphate in the oral environment, it has been suggested that it had a caries preventive effect [9-12]. In acidic conditions, by releasing free calcium and phosphate ions, it increases the calcium phosphate level in the plaque and, therefore, ensures the supersaturation in a way of preventing the demineralization and increasing remineralization. In order to prevent early caries lesions on patients undergoing orthodontic treatment, it is considered beneficial to use products containing CPP-ACP. It has been explained that CPP-ACP applied enamel surface being more resistance against acid-etching attacks might affect the bracket bonding [9-12]. In the literature, there are controversial reports on how CCP-ACP application changes bond strength [10-12]. All the studies in the literature, it has been determined that CPP-ACP treatment had been applied before the acid-etching process [10-12]. In a recently study, it has been concluded that CPP-ACPF application after acid-etching had considerably increased the shear bond strength of a total etch adhesive system and caused more cohesive fractures [9]. We thought that the studies conducted by using various bonding systems and preventive agents would contribute the literature. Therefore, the aim of this *in vitro* study was to investigate the effect of CPP-ACPF paste and fluorid varnish (5% NaF) application before/after acid etching on the bracket-enamel shear bond strength (SBS) of a total etch system and to compare them one self-etch system.

2. Materials and Methods

Ethical approval for this study was obtained from the Ethics Committee of the Dentistry School of the University of Necmettin Erbakan. A total sample size of 160 teeth (20 teeth per group) would give more than 80% power to detect significant differences with a 0.25 effect size between eight groups and at a p=0.05 significance level. A total of 160 upper premolar teeth extracted for orthodontic reasons from adolescent subjects (with the patient’s informed consent) were collected, stored in 0.5% Chloramine T solution at 4 °C and used within 3 months after extraction. Teeth with hypoplastic enamel, cracks, fractures, caries, restoration or pretreatment with a chemical agent such as alcohol, formalin, or hydrogen peroxide were excluded. Teeth were cleaned, and calculus-soft tissue remnants were removed. All teeth were mounted vertically in chemically activated acrylic resin until two-thirds of the root was embedded. Teeth polished with a nonfluoridated pumice and rubber prophylactic cup. The teeth were then rinsed in a stream of water for 10 s and dried. Teeth were divided into eight equal groups (20 teeth/group), two control and six test groups.

Group 1: This group served as a control for the two step etch-and-rinse adhesive system (Transbond XT, 3M Unitek, Monrovia, Calif). No pretreatment was applied on enamel. The enamel surface was acid-etched according to the manufacturers’ instructions with 37% phosphoric acid (Ultra Etch, Ultradent Products, INC) for 30 s, rinsed with water spray for 15s.

Group 2: The enamel was pre-treated with a fluoride-containing CPP–ACPF paste (MI Paste Plus; GC Europe, Leuven, Belgium) for 3 min, left undisturbed for 30 min (ref), then acid-etched as in group 1.

Group 3: The enamel was pre-treated with a fluoride-containing CPP–ACPF paste (as in group 2) after acid-etching.

Group 4: Enamel surfaces were pre-treated with %5 NaF (Enamel ProVarnish, Premier Dental, PA, Usa) for 4 min, left undisturbed for 3 min (ref), then acid-etched as group 1.

Group 5: Enamel surface was pre-treated with %5 NaF (as in group 4) after acid-etching.

Group 6: This group served as the control for the self-etching adhesive system (Transbond Plus, 3M Unitek). No pre-treatment was carried out. The brackets were bonded to the enamel with the self-etching adhesive system, Transbond Plus (3M Unitek), in accordance with the manufacturer’s recommendations. (ref)

Group 7: The enamel was pre-treated with a fluoride-containing CPP–ACPF paste (as in group 2) then self-etch adhesive system was used as in Group 6.

Group 8: Enamel surfaces were pre-treated with %5 NaF (as in group 4) then self-etch self-etch adhesive system was used as in Group 6.

After adhesive system application, a small amount of composite resin (Transbond XT) was placed onto the bracket, excess adhesive was removed from around the base of the bracket and the adhesive was then light-cured (Elipar S10, 3M ESPE, Seefeld, Germany) for 20 seconds on each interproximal side. After the bracket bonding procedure, all the bonded samples were stored in human saliva at 37°C for 1 day. To simulate the oral environment all samples thermocycled for 5000 cycles between 5°C and 55°C with a dwell time of 15 s in each water tank (Thermocycler, Willytec, Munich, Germany). All teeth were stored in distilled water at 37°C for 35 days before testing procedures. The water was changed weekly.

Shear bond strengths were measured on a universal testing machine (TSTM 02500, Elista Ltd, Şti, Istanbul, Turkey), at a crosshead speed of 0.5 mm/min. The results were recorded in newtons and converted to megapascals (MPa=N/mm2). Debonding surfaces were examined under a stereomicroscope (SZ-40 Olympus, Japan) at 40X magnification. The adhesive remnant index (ARI) system was used to classify failure types [13].

All statistical analyses were conducted with the Statistical Package for Social Sciences (SPSS 21.0 for Windows, SPSS Inc., Chicago, IL, USA). Statistical significance was set at a probability value of p<0.05. Since, the results of Kolmogorov-Smirnov and Shapiro-Wilk tests showed that the data were normally distributed (p>0.05), parametric tests were used for statistical analysis. Descriptive statistics including the mean, standard deviation, minimum, and maximum values were calculated for groups. The data were analyzed with two-way analyses of variance (ANOVA), and a post hoc Tukey’s test.

3. Results and Discussion

Mean shear bond strength values of the groups are listed in Table 1. ANOVA analysis showed significant differences among the eight groups (p<0.001). The highest and lowest SBS values were found in groups 3 and 4, respectively. The results of the post hoc Tukey test indicated that there were no significant differences between all control and test groups, except for Group 4 and 8. The mean SBS ranged from 7.48 MPa (Group 4) to 14.74 MPa (Group 3) for total etch groups. The mean SBS ranged from 7.92 MPa (Group 8) to 12.38 MPa (Group 6) for self-etch groups. For total etch groups, higher SBS means were obtained when the CPP-ACPF or 5% NaF were applied after acid-etching. The highest SBS means were ensured in total etch groups then self-etch adhesive groups, but these were not statistically significant (p>0.05). A

5% NaF groups showed the lowest SBS values compared to CPP-ACPF groups when applied before etching (Group 4 and 8).

The ARI scores for the groups tested are listed in Table 2. The G-test indicated that there were significant differences among the eight groups ($p<0.05$). Generally, more composite residues on enamel surfaces were obtained when CPP-ACPF or 5% NaF agents were applied after acid-etching. This was characterized by a alteration from ARI scores of 4 and 5 before acid-etching to ARI scores of 1, 2 and 3 after acid-etching.

Many preventive methods have been recommended to accomplish this issue such as using fluoride releasing adhesives and the use of preventive materials on enamel surface before bracket bonding [7-12]. The mechanism by which fluoride decreases demineralization and caries has also been shown to increase the resistance of enamel to acids. The fluoride deposits in hydroxyapatite form fluorapatite, which is claimed to affect the bond strength and/or debonded interface. Also, topically administer CPP–ACPF buffers free calcium and phosphate ion activity, maintaining a state of supersaturation with respect to tooth enamel that helps prevent demineralization and facilitates remineralization. This mechanism may interfere with acid-etching process [7-10].

In the present study, enamel surface treatment with %5NaF before acid-etching significantly decreased shear bond strength of bracket compared to the control. The use of fluoride during different stage of orthodontic bracketing is still questionable owing to its impact on SBS of orthodontic brackets [8]. Fluoride promotes the formation of fluorapatite in enamel, which is considered less soluble than hydroxyapatite. Teeth with high fluoride concentrations are generally considered more resistant to etching and may require more conditioning time [8, 9]. One study reported that topically administered fluorides could significantly reduce bond strength by distorting the creation of resin tags [14]. Also, other study suggested that administration of 5% NaF alone before acid-etching was found to decrease bracket SBS [15]. Cacciafesta *et al*. investigated the effect of fluoride application at 3 different steps of the bonding procedure (during enamel precleaning, before conditioning, and immediately before bonding) on SBS of a RMGIC and reported significantly lower SBS with fluoride application before or after acid-etching [16]. However, some studies have demonstrate that fluoride or fluoridated dentifrices do not seem to affect the SBS, the action of fluoride on the enamel should be considered because previous studies used different methodologies [17]. Other factors such as variation in the fluoride concentrations used, improvements in the properties of the bonding agents, and/or the bracket retention mechanism could also affect the results.

In the current study application of a higher concentration of 5% NaF before etching showed the lowest bond strength in two adhesive groups. One study suggested that the proportion of additional fluoride correlated significantly and negatively with the bond strength of orthodontic bracket [18]. Also, 5% NaF groups suggested the lowest SBS values compared to CPP-ACPF groups. One study reported that 5% NaF application showed the lowest SBS means compared to CPP-ACP and CPP-ACPF groups [9]. Lata *et al* reported that enamel treated with fluoride varnish was harder than enamel remineralised with CPP-ACP [19]. This might be connected to the variations in their reaction with tooth surface and their remineralisation activity or process. Furthermore, the distinction in the physical properties between the varnish and the paste might have some impacts. The varnish volatilized rapidly to create a weak film on enamel surface, while the paste might be clear away subsequent washing. One study suggested that minimum bond strength of 6 to 8 MPa was adequate for most clinical orthodontic needs [20]. These bond strengths are considered able to withstand masticatory and orthodontic forces. In this study, all bond strength values obtained were much above this minimal requirement.

According to our results, SBS increased (but not significantly) when CPP-ACPF was applied after acid-etching. To our knowledge, only one study evaluated the effects of CPP-ACPF or CPP-ACP after acid-etching process on bracket SBS [9]. Al-Kawari and Al-Jobair reported that in all experimental groups in their study, the bracket SBS values increased when the preventive agents were applied after acid-etching [9]. This is possible be connected to the fact that when CPP-ACP, CPP-ACPF and fluoride varnish were implemented before etching process, they promote the strength of dental enamel to acid, which can impress bracket adhesion and result in decreasing the bracket SBS means. One study suggested that topical application of CPP-ACP for remineralization of enamel subsurface lesions in situ with mineral that is more resistant to subsequent acid challenge [21]. Our results showed that there was no statistically significant difference in the SBS between the control and the CPP-ACPF groups when applied before or after acid-etching. This pointed out that the application of CPP-ACPF before or after acid-etching has no negative effect on bracket bonding strength. One study reported that application of CPP-ACP, either alone or combined with fluoride before acid-etching may safely be used as a prophylactic agent prior to bracket bonding [15]. Xiaojun *et al*. suggested that the use of CPP-ACP before acid-etching did not affect the bracket SBS [22]. Other study reported that the application of CPP-ACPF before acid-etching has no effect on bracket SBS when using self-etching systems [23]. This was in accordance with our results we found that the use of CPP-ACPF with self-etch adhesive decreased (but not significantly) SBS means. Contradictory to our result, one study suggested that the use of CPP-ACP alone or combined with fluoride has demonstrated significant increase in the SBS of orthodontic brackets [24]. In our study, lower SBS values were observed in the groups prepared with the self-etch adhesive system than in those bonded with phosphoric acid etching ($p\leq0.05$).

The ARI scores showed that lower ARI scores were recorded when the agents were applied after acid-etching, but this pattern did not different from control group. This was characterized by a shift from ARI scores of 3 and 4 before acid-etching to ARI scores of 1 and 2 after acid-etching. In the present study and others, the distribution of ARI scores were in accordance with the SBS values of the groups [9, 25, 26]. On the other hand, based on the ARI analysis, it was not possible to relate decrease of bond strength values with the ARI scores. According to the adhesive remnant index (ARI) system preferred in this study, the most wanted clinical condition is a high ARI score with no composite remaining on the tooth surface. Also Kimura *et al*., evaluating the effect of fluoride varnish, reported failures predominantly at the enamel/resin interface, which might be attributed to a disability in the penetration of the adhesive into the enamel [17].

3.1. Tables

Table 1: Descriptive statistics of shear bond strengths in MPa and comparison of the groups.

Groups		Mean	S.D.	ANOVA	Tukey
Etch and Rinse	1-Control	14.02	3.40	p<0.001	A
	2-CPP-ACPF before etch	13.35	3.79		A
	3-CPP-ACPF after etch	14.74	4.08		A
	4- 5% NaF before etch	7.48	2.90		B
	5- 5% NaF after etch	11.34	4.53		A
Self-Etch	6-Control	12.38	4.02	*F*=34.812	A
	7- CPP-ACPF	10.66	3.62		AB
	8- 5% NaF	7.92	2.67		B

Means with the same capital letter are not statistically different from each other (p>0.05).

Table 2: Frequency of distributions and comparison of ARI scores (%).

	N	1	2	3	4	5
1-Control	20	4 (20)	3 (15)	7 (35)	6 (30)	-
2-CPP-ACPF before etch	20	3 (15)	5 (25)	5 (25)	6 (30)	1 (5)
3-CPP-ACPF after etch	20	7 (35)	6 (30)	4 (20)	3 (15)	-
4- 5% NaF before etch	20	-	-	4 (20)	6 (30)	10 (50)
5- 5% NaF after etch	20	2 (10)	6 (30)	6 (30)	4 (20)	2 (10)
6-Control	20	3 (15)	5 (25)	4 (20)	4 (20)	4 (20)
7- CPP-ACPF	20	3 (15)	4 (20)	5 (25)	4 (20)	4 (20)
8- 5% NaF	20	2 (10)	2 (10)	6 (30)	6 (30)	4 (20)

ARI scores, 1: all of adhesive, with impression of bracket base, remained on enamel; 2: more than 90% of adhesive remained; 3: more than 10% but less than 90% of adhesive remained; 4: less than 10% of adhesive remained ; 5: no adhesive remained on enamel. ($p<0.05$)

Conclusions

Within the limitations of this *in vitro* study, brackets SBS was not changed significantly when CPP-ACPF applied after/before acid-etching. Significantly decreased SBS mean was obtained when %5NaF application was made before etching independently two adhesive systems. Further clinical studies to research the clinical effectiveness of these preventive agents before or after orthodontic bracket bonding would be required to verify these *in vitro* results.

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