

Effects of a fluoride-containing casein phosphopeptide–amorphous calcium phosphate complex on the shear bond strength of orthodontic brackets

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SUMMARY The purpose of this study was to investigate the effects of enamel pre-treatment with a new fluoride-containing casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) complex on the shear bond strength (SBS) of brackets bonded with etch-and-rinse or self-etching adhesive systems.

The material comprised 66 extracted human premolars randomly divided into six equal groups with respect to the enamel pre-treatment and adhesive system employed: 1. No pre-treatment and brackets bonded with the etch-and-rinse adhesive system (Transbond XT). 2. Pre-treatment with fluoride-containing CPP–ACP paste (MI Paste Plus) and Transbond XT. 3. Pre-treatment with non-fluoride CPP–ACP paste (MI Paste) and Transbond XT. 4. No pre-treatment and brackets bonded with the self-etching adhesive system (Transbond Plus). 5 and 6. Enamel pre-treated as for groups 2 and 3, respectively, and the Transbond Plus. Bonded specimens were subjected to thermal cycling ($\times 1000$) before SBS testing. The residual adhesive on the enamel surface was evaluated after debonding with the adhesive remnant index (ARI). Data evaluation was made using one-way analysis of variance and Tukey test for SBS results, and Kruskal–Wallis test for ARI results.

The results showed that enamel pre-treatment with either fluoride or non-fluoride CPP–ACP paste had no significant effect on the SBS of the self-etching adhesive system ($P > 0.05$). Enamel pre-treatment with non-fluoride CPP–ACP in group 3 significantly reduced the SBS of the etch-and-rinse adhesive ($P < 0.001$), while pre-treatment with fluoride-containing CPP–ACP paste (groups 2 and 5) did not affect debonding values ($P > 0.05$). The fluoride-containing CPP–ACP did not compromise the SBS of brackets bonded with the tested etch-and-rinse and self-etching systems, but its non-fluoride version significantly decreased the SBS of the etch-and-rinse adhesive system.

Introduction

The presence of archwires, brackets, and bands compromises oral hygiene practice in patients undergoing fixed orthodontic treatment. As such, white spot lesions (WSL), the precursor of enamel caries, can frequently be seen due to the prolonged accumulation and retention of bacterial plaque adjacent to fixed orthodontic appliances (Øgaard *et al.*, 1988a,b; Chadwick *et al.*, 2005). In order to improve oral hygiene and prevent WSL formation, several methods have been suggested in orthodontic practice. A plethora of studies (Stratemann and Shannon, 1974; O'Reilly and Featherstone, 1987; Geiger *et al.*, 1988; Øgaard *et al.*, 1988a,b; Øgaard, 1989; Adriaens *et al.*, 1990) have shown that various forms of topically applied fluoride before (Lehman and Davidson, 1981; Wang and Sheen, 1991), during (Bohrer and Gedalie, 1980; Freeman and Shannon, 1981), or after (Davidson and Bekke-Hoekstra, 1980) etching of enamel for bracket bonding can reduce or

eliminate decalcification during orthodontic treatment. Other frequently recommended measures against WSL formation include the use of chlorhexidine rinses and fissure sealants (Polat *et al.*, 2005; Heinig and Hartmann, 2008).

More recently, a milk protein derivative, casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) has been recommended for caries prevention and enamel remineralization (Heinig and Hartmann, 2008). Topically administered CPP–ACP buffers free calcium and phosphate ion activity, maintaining a state of supersaturation with respect to tooth enamel that helps prevent demineralization and facilitates remineralization (Heinig and Hartmann, 2008). Thus, a wide spectrum of potential applications of CPP–ACP preparations, including prevention and treatment of WSL in orthodontics, has been recommended (Reynolds *et al.*, 1995; Reynolds, 1997). Both laboratory and *in situ* studies have shown that CPP–ACP can remineralize human enamel subsurface lesions

(Reynolds *et al.*, 1995; Reynolds, 1997; Rose, 2000a,b). On the other hand, enamel remineralized with CPP-ACP has been shown to be more resistant to acids (Iijima *et al.*, 2004), which may interfere with resin bonding. Such adverse effects may be more pronounced with the use of the new version of the CPP-ACP complex, which contains 900 ppm sodium fluoride. Enamel pre-treatment with fluoride can also interfere with the bonding mechanism of resins, resulting in reduced bond strength (Meng *et al.*, 1998).

Consequently, the aim of this study was to investigate and compare the effects of fluoride-containing CPP-ACP paste versus its non-fluoride version on the shear bond strength (SBS) of orthodontic brackets bonded with etch-and-rinse or self-etching adhesive systems. The null hypothesis tested was that pre-treatment of enamel with the new fluoride-containing CPP-ACP paste would significantly decrease the SBS of both types of orthodontic adhesive systems.

Materials and methods

Specimen preparation

Human premolars, recently extracted for orthodontic purposes from adolescents, were used in the present study. The teeth were taken from individuals living in a non-fluoridated area. After extraction, the teeth were stored in distilled water and were subjected to testing approximately 6 months after collection. No sterilization of the teeth was undertaken prior to testing. Tooth selection criteria included absence of caries or cracks when inspected under visible light at $\times 4$ magnification. After cleaning the enamel surfaces with a non-fluoridated pumice, the 66 selected teeth were randomly divided into six equal groups with respect to the enamel pre-treatments and adhesive systems employed:

Group 1: This group served as the control for the etch-and-rinse adhesive system. No pre-treatment was carried out. The enamel surface was etched with 37 per cent phosphoric acid for 30 seconds, rinsed with a water spray for 15 seconds, and air-dried. Metal brackets (Microarch Standart; GAC International, Bohemia, New York, USA) were bonded with the etch-and-rinse adhesive system, Transbond XT (3M Unitek, Monrovia, California, USA), in accordance with the manufacturer's instructions.

Group 2: The enamel was pre-treated with a fluoride-containing CPP-ACP paste (MI Paste Plus; GC Europe, Leuven, Belgium). The enamel was acid-etched as in group 1. The brackets were bonded with the etch-and-rinse adhesive system, Transbond XT.

Group 3: The enamel was pre-treated with a non-fluoride CPP-ACP paste (MI Paste; GC Europe). Etching of the enamel and bracket bonding was performed as in groups 1 and 2.

Group 4: This group served as the control for the self-etching adhesive system. 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.

Group 5: The enamel was pre-treated with the fluoride-containing CPP-ACP paste and the brackets were bonded to the enamel with the self-etching adhesive system, Transbond Plus.

Group 6: The enamel was pre-treated with a non-fluoride CPP-ACP paste. The brackets were bonded to the enamel with Transbond Plus.

The adhesives were light cured with a quartz-tungsten-halogen source (Hilux; Benlioglu Dental, Istanbul, Turkey) with a wavelength of 560 mW/cm² from both the mesial and the distal aspects of the brackets for 20 seconds each. Before photopolymerization, the irradiance of the light curing unit was confirmed with a built-in radiometer. Bonded specimens were first stored in deionized water at 37°C for 24 hours and then subjected to thermal cycling in deionized water at $5 \pm 2^\circ\text{C}$ – $55 \pm 2^\circ\text{C}$ for 1000 cycles with a dwell time of 5 seconds. To facilitate degradation of bonds, the specimens were further stored in distilled water at room temperature for 6 weeks before debonding. The water was changed weekly (Kitasako *et al.*, 2000; Çehreli *et al.*, 2005).

SBS testing

Following water storage and thermal agitation, the roots were removed with a slow-speed diamond saw under water coolant. The crowns were embedded in self-curing acrylic resin within a mounting jig that aligned the labial surface of each tooth parallel to the direction of a shearing force. A universal testing device (model 4204; Instron, Canton, Massachusetts, USA) was used to apply shearing force in an occlusogingival direction to the bracket base at a crosshead speed of 1 mm/minute. The force required to dislodge the bracket was recorded in newtons (N) and converted to megapascals (MPa) using the following formula: bond strength (MPa) = debonding force (N)/[w \times l] (mm²), where w = width of the bracket base, l = height of the bracket base, and 1 MPa = 1 N/mm². The orthodontic metal brackets had a base area of 12 mm (Øgaard *et al.*, 1988a,b).

Following debonding, the teeth and brackets were examined under a stereomicroscope (Carl Zeiss Inc., Oakland, California, USA) at $\times 10$ magnification to determine any adhesive remaining, in accordance with the modified adhesive remnant index (ARI; Olsen *et al.*, 1997). ARI scores range from 5 to 1: 5 = no adherence of composite on the enamel, 4 = less than 10 per cent of the composite remaining on the enamel, 3 = more than 10 per cent but less than 90 per cent of the composite remaining on the enamel, 2 = more than 90 per cent of the composite remaining on the enamel, and 1 = all composite remaining on the enamel, with the impression of the bracket base.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences 11.5 software (SPSS Inc.,

Chicago, Illinois, USA). Data were expressed as the mean \pm the standard deviation for the SBS levels and median (minimum–maximum) for ARI scores, respectively. The mean bond strength differences among groups were analysed using one-way analysis of variance *post hoc* Tukey test, and the Kruskal–Wallis test for evaluation of ARI scores. A *P* value less than 0.05 was considered statistically significant.

Results

The SBS values and ARI scores of the test groups are presented in Table 1. The highest SBS values were observed when the brackets were bonded to untreated (intact) enamel (groups 1 and 4), with Transbond XT and Transbond Plus yielding similar SBS values ($P < 0.001$). Pre-treatment of enamel with the fluoride-containing CPP-ACP paste did not affect the SBS of Transbond XT, which showed similar debonding values with both control groups ($P < 0.001$). On the other hand, enamel pre-treatment in group 3 with the non-fluoride CPP-ACP paste significantly reduced the SBS ($P < 0.001$). Enamel pre-treatment in groups 5 and 6 with either the fluoride or the non-fluoride CPP-ACP paste had no significant effect on the SBS of the brackets bonded with the self-etching adhesive system, Transbond Plus ($P > 0.05$).

There were no significant differences among the ARI scores of test groups (Table 1, $P > 0.05$). The lowest ARI score was observed in group 1. Compared with their controls, both adhesive systems displayed higher (but statistically insignificant) ARI scores on enamel pre-treated with CPP-ACP pastes.

Discussion

An increasing number of laboratory and animal studies indicate the strong anticarcinogenic potential of CPP-ACP-based compounds (Reynolds, 1997; Rose, 2000a,b). Calcium and phosphate are essential components of enamel in the form of highly insoluble complexes, but in the presence of CPP, they remain soluble and biologically available on enamel and even in dental plaque (Llena *et al.*, 2009). This provides a large reservoir of calcium and phosphate ions (Reynolds, 2008), which inhibit demineralization and favour remineralization under cariogenic attack (Llena *et al.*, 2009). The latter effect has recently been confirmed on WSL by clinical scoring and laser fluorescence assessment (Andersson *et al.*, 2007) indicating that the proprietary compounds of CPP-ACP not only promote regression of WSL after debonding of fixed orthodontic appliances but can also result in the disappearance of 63 per cent of WSL sites after a 12 month clinical CPP-ACP regimen.

Kumar *et al.* (2008) demonstrated that CPP-ACP showed higher remineralization potential when used in combination with fluoride toothpastes. Furthermore, experimental incorporation of fluoride into CPP-ACP complexes has

Table 1 Mean and standard deviations (SD) of the shear bond strength (SBS) and adhesive remnant index (ARI) scores of the test groups.

Groups	SBS value (MPa)			ARI scores Median (minimum–maximum)
	Mean	SD	Standard error	
1. Transbond XT	8.88 ^a	1.61	0.49	3 (3–5)
2. MI Paste Plus + Transbond XT	8.82 ^b	1.54	0.46	4 (3–5)
3. MI Paste + Transbond XT	5.74 ^{a,b,c,d}	1.67	0.50	5 (2–5)
4. Transbond Plus	9.08 ^c	1.45	0.44	4 (3–5)
5. MI Paste Plus + Transbond Plus	8.11	1.59	0.48	5 (5–5)
6. MI Paste + Transbond Plus	7.33 ^d	2.20	0.66	5 (3–5)
<i>P</i> value	<0.001*			0.055**

*One-way analysis of variance.

**Kruskal–Wallis test.

Values with the same superscript letters indicate significant differences at $P < 0.001$.

been shown to provide significantly greater levels of remineralization *in situ* and *in vitro* (Reynolds *et al.*, 2008) compared with those achieved with CPP-ACP or fluoride alone (Reynolds *et al.*, 2008). Thus, incorporation of fluoride in proprietary CPP-ACP compounds appears to be a logical step towards achieving the synergistic effect of CPP-ACP and fluoride in reducing caries experience. On the other hand, the potential adverse effect of fluoride pre-treatment on resin bonding (Gwinnett *et al.*, 1972; Sheykholeslam *et al.*, 1972; Meng *et al.*, 1998) justifies the present research to investigate the effects of caries prophylaxis with the 900 ppm fluoride-containing CPP-ACP compound on the SBS of orthodontic brackets.

In the present study, both the etch-and-rinse and the self-etch adhesive systems showed a slight but statistically insignificant reduction in SBS to fluoride-containing CPP-ACP pre-treated enamel. Although further research on the mechanism causing this decrease is warranted, these results necessitate rejection of the null hypothesis since enamel pre-treatment with the fluoride-containing CPP-ACP compound did not compromise the bond strength of the tested etch-and-rinse and self-etch adhesive systems. Two reasons might be responsible for these findings: firstly, the sodium fluoride present in the fluoride-containing CPP-ACP paste could interact with the ACP component of the casein complex, rendering both inorganic components ineffective (Azarpazhooh and Limeback, 2008; Rehder Neto *et al.*, 2009). Undoubtedly, the experimental nature of the present study limits verification of this assumption, which merits further research. Secondly, the fluoride present in the CPP-ACP compound might have actually been deposited on the enamel surface as nanocomplexes (Cross *et al.*, 2004), but such fluoride pre-treatment of intact surface enamel may have little if any adverse effect on the SBS of the tested adhesives. Indeed, a number of laboratory

studies have indicated that fluoride prophylaxis of enamel before bracket bonding does not necessarily reduce the bond strength of orthodontic adhesive resins (Bishara *et al.*, 1989; Wang and Sheen, 1991; Garcia-Godoy, 1993). Accordingly, pre-treatment of enamel with the tested fluoride-containing CPP-ACP complex appears to be a safe prophylactic (or therapeutic) procedure in terms of bracket bond strength achieved with the present adhesive systems.

Based on two recent studies that evaluated the effects of non-fluoridated CPP-ACP formulations on orthodontic bond strength (Kecik *et al.*, 2008; Xiaojun *et al.*, 2009), a proprietary non-fluoridated CPP-ACP paste was utilized for comparative purposes. The present results showed that the non-fluoride CPP-ACP paste did not affect the SBS of the self-etching adhesive system, Transbond Plus, and that the SBS value was similar to those achieved with both adhesive systems on enamel pre-treated with the fluoride-containing CPP-ACP. These results corroborate the findings of previous studies, which demonstrated that CPP-ACP pre-treatment does not alter the SBS of the etch-and-rinse adhesive system, Transbond XT, to bovine enamel (Kecik *et al.*, 2008) or of the chemically cured Unite bonding adhesive system to human enamel (Xiaojun *et al.*, 2009). On the other hand, further studies are required to elucidate the mechanism behind the present low SBS value of Transbond XT to non-fluoride CPP-ACP-treated human enamel. In the light of recent data (Adebayo *et al.*, 2007), non-fluoride CPP-ACP treatment of enamel does not appear to decrease the micro-SBS of etch-and-rinse and self-etching adhesives, suggesting that the low SBS value of Transbond XT might not merely be a result of the testing method, substrate material, or operator variability (Adebayo *et al.*, 2008).

Conclusions

Within the experimental limitations of the present study, the following conclusions were drawn:

1. The fluoride-containing CPP-ACP compound did not compromise the bond strength of brackets bonded with the tested etch-and-rinse and self-etching systems. Moreover, the non-fluoride version of the CPP-ACP compound did not alter the SBS of the self-etching system, yielding bond strength values similar to the latter two groups.
2. Enamel pre-treatment, with the non-fluoride version of the CPP-ACP paste, decreased the SBS of Transbond XT to a value lower than the 6–8 MPa bond strength value recommended by Reynolds (1975) and Whitlock *et al.* (1994) as adequate for orthodontic purposes. Based on this result, the use of Transbond XT on non-fluoride CPP-ACP pre-treated enamel cannot be recommended.

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