European Journal of Orthodontics 33 (2011) 282–287
doi:10.1093/ejo/cjq062
Advance Access Publication 22 July 2010

A comparative evaluation of casein phosphopeptide-amorphous calcium phosphate and fluoride on the shear bond strength of orthodontic brackets

Anis Tabrizi* and Banu Cakirer**
*Private Practice, Dubai, United Arab Emirates and **Department of Orthodontics, Faculty of Dentistry, Marmara University, Istanbul, Turkey

Correspondence to: Dr Banu Cakirer, Ahmet Fetgari sok, Trakya Apt. 16/3, Tesvikiye, 34365 Istanbul, Turkey.
E-mail: bcakirer@marmara.edu.tr

SUMMARY The purpose of the study was to evaluate and compare the effects of topical application of casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) and fluoride on the shear bond strength (SBS) of orthodontic brackets bonded to human premolar teeth.

Eighty extracted human premolar teeth were cleansed of soft tissue, then polished with non-fluoridated pumice, and immersed in water for 1–3 months until testing. The teeth were randomly divided into four groups: group 1, control, without pre-treatment of the enamel; group 2, the enamel was treated with 5 per cent sodium fluoride varnish for 4 minutes; group 3, the enamel was treated with CPP-ACP for 3 minutes; and group 4, the enamel was treated with 5 per cent sodium fluoride and CPP-ACP. All bonded specimens were immersed in distilled water at room temperature for 24 hours and subsequently tested for SBS in a universal testing machine. After debonding, the bracket bases and the enamel surfaces were examined by eye to assess any adhesive remaining, in accordance with the Adhesive Remnant Index (ARI). Descriptive statistics were calculated for each group. Analysis of variance and Tukey honestly significant difference (HSD) test were performed to compare the SBS of the groups. The chi-square test was used to evaluate differences in ARI scores between the groups.

The SBS in group 2 was significantly lower than groups 1, 3, and 4 ($P < 0.001$). ARI scores were not significantly different between the four groups ($P > 0.05$). CPP-ACP, either alone or combined with fluoride, may safely be used as a prophylactic agent before bracket bonding.

Introduction

The demineralization of enamel adjacent to orthodontic brackets is a significant clinical problem because orthodontic appliances tend to hinder plaque removal and create an environment in which the absence of good oral care can lead to rapid demineralization. In a cross-sectional study, Gorelick *et al.* (1982) reported that 50 per cent of individuals undergoing fixed orthodontic treatment had a non-developmental white spot lesion (WSL) compared with 25 per cent of controls.

Emphasis should be placed on controlling dental plaque before the orthodontic appliances are placed (Benson *et al.*, 2005). Prophylactic chemical control can be performed when mechanical control is not effective. Fluoride is important in the prevention of enamel demineralization (Margolis and Moreno, 1990; Benson *et al.*, 2005). It has been reported that topical fluoride application induces deposition of calcium-fluoride-like material (CaF_2) on the enamel surface, which is effective in increasing the resistance to dental caries or enamel demineralization (Ögaard, 2001; Gontijo *et al.*, 2007). Fluoride varnish has high fluoride concentrations and its application technique is simple and fast. The varnish is not affected by humidity, remains adhered to enamel during a significant period of time, and its use does not require patient cooperation (Petersson, 1993; Sëppa *et al.*, 1994). All these characteristics indicate that varnish application may easily be incorporated into the daily clinic routine (Todd *et al.*, 1999). Different methods of applying topical fluoride include application of the fluoride before etching, incorporation of fluorides in etching solutions, topical application of fluorides to the etched enamel surfaces before bonding, and topical applications of the fluorides after bonding. However, some studies have shown controversial results regarding the effect of topical application of fluoride on bracket bond strength (Bishara *et al.*, 1989; Garcia-Godoy *et al.*, 1991; Wang and Sheen, 1991; Meng *et al.*, 1997, 1998; Kim *et al.*, 2005).

Although the orthodontic specialty has focused mainly on protocols for fluoride intervention, another mechanism has been shown to favour remineralization dynamics: casein phosphopeptides (CPPs)-stabilized calcium phosphate solutions were shown to remineralize subsurface lesions (Reynolds, 1997). The anticariogenic properties of milk and milk products, such as cheese, have been studied previously in animal models (Reynolds and Johnson, 1981; Rosen *et al.*, 1984). This activity has been attributed to the direct chemical effects of phosphoprotein casein and calcium phosphate components in cheese (Harper *et al.*, 1986; Reynolds, 1997). Calcium and phosphate are essential components of enamel and dentine and form highly insoluble complexes, but in the presence of CPPs, they remain soluble and biologically available (Llena *et al.*, 2009). It has been suggested that CPPs stabilize soluble calcium phosphate by binding amorphous calcium phosphate (ACP) with their multiple phosphoserine residues, thereby allowing the formation of small CPP-ACP clusters (Reynolds, 1997). CPP-ACP might prevent tooth erosion by suppressing demineralization, enhancing remineralization, or a combination of these two processes. Recently, it was reported that the inorganic components contained in high concentrations of CPP-ACP were able to act to enhance remineralization of the enamel structure (Yamaguchi *et al.*, 2006; Oshiro *et al.*, 2007). This CPP–ACP complex applied to teeth by means of chewing gum, toothpaste, lozenges, mouth rinses, or sprays is able to adhere to the dental biofilm and enamel hydroxyapatite providing bioavailable calcium and phosphate ions (Llena *et al.*, 2009). Since its introduction in 2002, the use of Tooth Mousse™ (a CPP-ACP) as a topical coating for teeth has been gaining popularity, especially among paediatric dental professionals not only because of its remineralization effect on WSL but also because of its caries-preventing effect in long-term clinical use (Yengopal and Mickenautsch, 2009). Application of CPP-ACPs has a similar effect to self-applied fluorides and has been shown to reduce the appearance of new carious lesions in patients with xerostomia and also to enhance fluoride activity (Llena *et al.*, 2009).

Today, an increasing number of patients attend for orthodontic consultation whose teeth have been coated with CPP-ACP as a prophylactic agent, before orthodontic treatment. However, the data reporting the effects of CPP-ACP on bracket bonding are limited (Keçik *et al.*, 2008).

Therefore, the aim of this study was to evaluate and compare the effects of topical application of CPP-ACP, fluoride, and simultaneous application of CPP-ACP and fluoride on the shear bond strength (SBS) of orthodontic brackets bonded to human premolar teeth.

Materials and methods

The study was carried out in the Research Laboratory of Selçuk University School of Dentistry with the permission of the local Research Committee.

Eighty human premolars were collected from patients whose teeth were extracted for orthodontic treatment purposes. The extracted teeth were cleansed of soft tissue and then polished with non-fluoridated pumice and rubber prophylactic cups at a low speed for 10 seconds and immersed in distilled water in a sealed container for 1–3 months until testing. The water was changed weekly, to prevent the growth of microorganisms. The criteria for tooth selection included (1) intact buccal enamel with no cracks caused by the pressure of the extraction forceps, (2) no caries, and (3) buccal enamel surfaces, which had not been treated with hydrogen peroxide (H_2O_2), formalin, alcohol, or other chemical agents after extraction.

The teeth were randomly assigned to one of four groups ($n = 20$):

Group 1: control without pre-treatment of the enamel
Group 2: the enamel was treated with 5 per cent sodium fluoride varnish (Duraphat Colgate Oral Pharmaceuticals, Inc., Canton, Massachusetts, USA) for 4 minutes.
Group 3: the enamel was treated with CPP-ACP (Recaldent Tooth Mousse; GC Europe, Leuven, Belgium) for 3 minutes
Group 4: the enamel was treated with 5 per cent sodium fluoride for 4 minutes and with CPP-ACP for 3 minutes.

The treated teeth were left for 30 minutes before the CPP-ACP ± the fluoride was washed off with a 1 minute water spray, as described by Wang and Sheen (1991).

The roots of the teeth were embedded in a cubic mould using chemically cured acrylic resin (Vertex, Zeist, The Netherlands) with a mounting jig used to align the buccal surface of each tooth so that it was perpendicular to the base of the mould.

After the buccal surfaces of each tooth were etched with 37 per cent phosphoric acid gel for 15 seconds, they were rinsed with a water spray for 30 seconds and dried with an oil-free air source for 20 seconds. Orthodontic metal brackets (0.018 inch slot; MIB, Rueil, France) with a base area of approximately 10.38 mm^2 were bonded with the same adhesive (Light Bond and Composite; Reliance, Orthodontic Products, Inc., Itasca, Illinois, USA). The base area of the brackets was measured by the same researcher (AT). The brackets were positioned on the centre of the buccal surfaces with sufficient pressure to express excess adhesive, which was removed from the margins of the bracket base with a scaler before polymerization. All brackets were light-cured for 40 seconds with a halogen light-curing unit (Hilux; Benlioglu, Istanbul, Turkey), 10 seconds each from the mesial, distal, occlusal, and gingival margins.

After bonding, all specimens were immersed in distilled water at room temperature for 24 hours and subsequently tested in shear mode on a universal testing machine (TSTM 02500; Elista Ltd Sti., Istanbul, Turkey). For shear testing, the specimens were secured in the lower jaw of the machine so that the bracket base of the sample paralleled the direction of the shear force. The specimens were stressed in an occlusogingival direction with a crosshead speed of 1 mm/minute (Jobolia *et al.*, 1997; Sfondrini *et al.*, 2001). The maximum load necessary to debond each bracket was recorded in Newton and then converted into megapascals as a ratio of Newton to surface area of the bracket.

After bond failure, the bracket bases and the enamel surfaces were examined by the same operator (AT) with the naked eye to assess any adhesive remaining on the tooth, in accordance with the Adhesive Remnant Index (ARI; Oliver, 1988; Cozza *et al.*, 2006; Montasser and Drummond, 2009). The rating assigned to each tooth was 1 = all adhesive remaining on the enamel surface; 2 = more than 50 per cent of the adhesive remaining on the tooth or less than 50 per cent remaining on the bracket; 3 = more than 50 per cent of the adhesive remaining on the bracket or less than 50 per cent of the adhesive remaining on the tooth; and 4 = no composite remaining on the tooth or all remaining on the bracket surface.

The most desired situation is a low ARI score with all the composite remaining on the enamel surface; thus, the likelihood of enamel fracture during debonding decreases when ARI scores are consistently low.

Results

Descriptive statistics, including the mean, standard deviation (SD), minimum and maximum values of the SBS for each of the four groups, and the results of the analysis of variance (ANOVA) comparing the SBSs (in megapascals) are presented in Table 1.

The results revealed that the mean SBS values were 21.0 MPa (SD 5.2), 14.0 MPa (SD 4.64), 22.5 MPa (SD 4.3), and 21.6 MPa (SD 3.5) for groups 1, 2, 3, and 4, respectively. ANOVA indicated that there was a significant difference among the shear bond values of the test and control groups ($P < 0.001$, Table 1).

Tukey test showed that shear bond values of groups 1, 3, and 4 were not statistically significantly different from each other ($P > 0.05$), while in group 2, treated with fluoride, these were statistically significantly lower SBS values ($P < 0.001$, Table 2).

The residual adhesive on the enamel surfaces, as indicated by ARI scores, are presented in Table 3. The results of the chi-square comparisons ($\chi^2 = 5.96$, $P = 0.427$) show that regarding the location of separation, there were no significant differences between the four groups tested. All groups showed a higher percentage of ARI scores of 3 and 4, which indicated that debonding distribution failures were mainly at the adhesive–enamel interface.

Discussion

Controlling dental plaque before and during fixed orthodontic treatment, without compromising bracket bond strength, has always been an area of research in orthodontics. Fluoride has been shown to be a prophylactic agent to prevent enamel demineralization before and during orthodontic treatment. However, controversial results as to the effect of topical application of fluoride on bracket bond strength (Bishara *et al.*, 1989; Garcia-Godoy *et al.*, 1991; Wang and Sheen, 1991; Meng *et al.*, 1997, 1998; Kim *et al.*, 2005) have been reported. The results of the present study suggest that fluoride varnish significantly decreases the bond strength of orthodontic brackets to enamel. This is in agreement with previous studies which showed that the topical application of fluoride can interfere with the etching effect of phosphoric acid on enamel surfaces, resulting in reduced bond strength of dental resins (Garcia-Godoy *et al.*, 1991; Meng *et al.*, 1997, 1998; Cacciafesta *et al.*, 2005). The benefits of fluoride in the prevention of tooth decay and remineralization of decalcified enamel have been described by Bibby (1942). Fluorides react with the enamel surface to form calcium fluoride and fluorapatite, making the surface more resistant to demineralization and decay. Gwinnett and Smith (1982), who reported that topically applied fluorides could significantly reduce bond strength by disrupting the formation of enamel tags, recommended the application of prophylactic agents prior to acid etching of the enamel. In the present study, although fluoride was applied before acid etching of the enamel, the results were consistent with the findings of Cacciafesta *et al.* (2005) who showed significantly lower bond strengths with fluoride application before etching or before bonding. Although in that study the teeth were immersed in 1.1 per cent acidulated phosphate fluoride (APF) gel, which was lower than the fluoride concentration used in the current study (5 per cent sodium fluoride), the

Table 1 Descriptive statistics (in megapascals) of the shear bond strength (SBS) of the four groups ($n = 20$ specimens per group) and the results of analysis of variance comparing the SBS values of the groups (F, fluoride; CPP-ACP, casein phosphopeptide-amorphous calcium phosphate).

Group	Minimum	Maximum	Mean	Standard deviation
1. control	13.89	33.33	21.02	5.24
2. F	5.22	22.73	14.02	4.64
3. CPP-ACP	13.49	29.57	22.57	4.32
4. F and CPP-ACP	17.26	30.95	21.69	3.57
F			15.30	
P			<0.001	

Table 2 Results of Tukey's multiple comparison test comparing the shear bond strengths of the groups (F, fluoride; CPP-ACP, casein phosphopeptide-amorphous calcium phosphate).

Compared groups	*P*
Group 1 (control)/group 2 (F)	***
Group 1(control)/group 3 (CPP-ACP)	NS
Group 1 (control)/group 4 (F+CPP-ACP)	NS
Group 2 (F)/group 3 (CPP-ACP)	***
Group 2 (F)/group 4 (F+CPP-ACP)	***
Group 3 (CPP-ACP)/group 4 (F+CPP-ACP)	NS

NS, not significant. ***$P < 0.001$.

Table 3 Frequency of distribution of Adhesive Remnant Index (ARI) scores and chi-square comparison of the four groups tested (F, fluoride; CPP-ACP, casein phosphopeptide-amorphous calcium phosphate).

ARI scores	1	2	3	4	χ^2
	n (%)	*n* (%)	*n* (%)	*n* (%)	
1. Control	0 (0.00)	2 (10.00)	10 (50.00)	8 (40.00)	
2. F	0 (0.00)	0 (0.00)	10 (50.00)	10 (50.00)	
3. CPP-ACP	0 (0.00)	1 (5.00)	13 (65.00)	6 (30.00)	5.96
4. F and CPP-ACP	0 (0.00)	3 (15.00)	12 (60.00)	5 (25.00)	NS

NS, not significant.

effect of fluoride application on the SBS of brackets was similar. Teeth with a higher concentration of fluoride are generally considered to be more resistant to acid etching and can require a longer etching time. The bond strengths of mild to moderately fluorosed teeth demonstrated a 40 per cent reduction compared with normal teeth (Opinya and Parmeijer, 1986). In the current study, application of a higher concentration of sodium fluoride (5 per cent sodium fluoride varnish; Duraphat) may explain the lower bond strength, with residual varnish hindering the full effect of the etch solution. Nevertheless, this displayed a bond strength twice that of the minimum (6–8 MPa) suggested by Reynolds (1975) as sufficient for clinical purposes.

There are a number of studies where the findings are contrary to the present results (Bishara *et al.*, 1989; Wang and Sheen, 1991; Damon *et al.*, 1996; Kimura *et al.*, 2004; Keçik *et al.*, 2008). The conflicting findings may be the result of a number of factors, such as the difference in application time, variation in the fluoride concentrations used, improvements in the properties of the bonding agents, and/or bracket retention mechanism, and thermocycling.

Although studies evaluating the effect of fluoride application on SBS can easily be found in the literature, data reporting the effects of CPP-ACP on bracket bonding is limited (Keçik *et al.*, 2008). Since there is an increasing number of patients whose teeth have previously been coated with CPP-ACP as a prophylactic agent before orthodontic treatment, it is important to have evidence-based data regarding the effects of CPP-ACP on the bond strength of brackets.

The dental specialty has focused mainly on protocols for fluoride intervention and the incorporation of fluoride into dental restorative materials. However, another mechanism can also favour remineralization dynamics: CPPs were shown to stabilize ACP, to localize ACP in dental plaque, and were anti-cariogenic in animal and *in situ* human caries models (Reynolds and Johnson, 1981; Rosen *et al.*, 1984; Harper *et al.*, 1986; Reynolds, 1997; Yamaguchi *et al.*, 2006; Oshiro *et al.*, 2007). ACP-filled methacrylate composites have also demonstrated the potential to remineralize carious enamel lesions (Skrtic *et al.*, 1996). The first commercially available ACP-containing orthodontic resin cement received Food and Drug Administration approval in 2002 and the results of a study by Dunn (2007) were published, which compared the SBS of orthodontic brackets bonded with enamel using a commercially available orthodontic adhesive containing ACP to that of brackets bonded with a conventional resin-based orthodontic adhesive. Under the conditions of that *in vitro* study, Dunn (2007) found that orthodontic brackets bonded to teeth with an ACP-containing composite material failed at significantly lower forces than those bonded to teeth with conventional resin-based composite orthodontic cement. Previous investigators have suggested that ACP-containing dental materials should be limited to situations where mechanical demands are less, such as in pit and fissure sealants or bases and liners (Park *et al.* 1998; Skrtic *et al.*, 2004).

The results of the present study showed that the CPP-ACP application or combined application of CPP-ACP and fluoride did not significantly affect SBS. It is suggested that CPP-ACP either alone or combined with fluoride may safely be used as a prophylactic agent before bracket bonding.

The findings of the present study are in agreement with those of Keçik *et al.* (2008) who compared the effects of topical application of CPP-ACP, APF, or both on the SBS of brackets bonded to bovine permanent mandibular incisors. Although it has been reported that bovine teeth could be a suitable alternative to human teeth in bond strength tests for primary dentition (Krifka *et al.*, 2008), precautions have been recommended in substitution of human substrate in laboratory studies (Schilke *et al.*, 1999; Abuabara *et al.*, 2004). As discussed by Keçik *et al.* (2008), bovine enamel and dentine develop more rapidly during tooth formation and bovine enamel has larger crystal grains and more lattice defects than human enamel (Moriwaki *et al.*, 1968), therefore reported lower critical surface tension in bovine than in human enamel may have been due to this developmental difference (Opinya and Parmeijer, 1986). These differences might have contributed to the SBS values found in the present study, which were larger than those reported by Keçik *et al.* (2008). Reynolds (1975) suggested that a minimum bond strength of 6–8 MPa was adequate for most orthodontic needs. These bond strengths are considered to be able to withstand masticatory and orthodontic forces. In the present study, although there was significant differences among the groups, all SBS values achieved were above this minimum requirement.

The ARI scores indicated no significant differences among the groups regarding the location of separation, which all showed a higher prevalence of ARI scores of 3 and 4 meaning that the bond between bracket and resin was stronger than that between the resin and enamel.

In the current study, every effort was made to standardize the testing procedure in an attempt to create a laboratory technique which is as representative of the clinical situation. The methodology used was largely based on previous studies (Fox *et al.*, 1994; Eliades *et al.*, 2000). However, it is acknowledged that *in vitro* bond strength testing is not truly representative of the highly demanding intraoral conditions and at best gives only an indication of possible clinical performance of the material tested. In spite of these limitations, the results still assist in determining which products should be taken to the next level of research. The validity of the results depends on how appropriate and representative the laboratory test conditions are and although in the present study each step of the protocol was strictly followed, the findings should be interpreted with caution.

Conclusions

The findings led to the following conclusions:

1. CPP-ACP may safely be used as a prophylactic agent before brackets are bonded, in an attempt to reduce the risk of demineralization during fixed orthodontic treatment.
2. Although 5 per cent sodium fluoride varnish application before etching was found to reduce the SBS of orthodontic brackets compared with CPP-ACP or CPP-ACP and fluoride application, all bond strength values were above the minimum clinical value. Thus, all the combinations tested showed clinically acceptable bond strengths.
3. No significant differences were found among the ARI scores of the groups.

Acknowledgements

We would like to thank Drs Serdar and Aslihan Usumez for their valuable supervision in the laboratory.

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