Original Article

Effect of fluoridated casein phospopeptide-amorphous-calcium phosphate complex, chlorhexidine fluoride mouthwash on shear bond strength of orthodontic brackets: A comparative *in vitro* study

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Abstract

Objective: The aim of the current study was to determine the effects of casein phosphopeptide amorphous calcium-phosphate (CPP-ACP) complex, chlorhexidine fluoride mouthwash on shear bond strengths (SBSs) of orthodontic brackets. **Materials and Methods:** About sixty extracted healthy human premolar teeth with intact buccal enamel were divided into two equal groups to which brackets were bonded using self-etching primers (SEPs) and conventional means respectively. These were further equally divided into three subgroups - (1) control (2) CPP-ACP (3) chlorhexidine fluoride mouthwash. The SBSs were then measured using a universal testing machine. **Results:** SBS of the conventional group was significantly higher than the self-etching group. The intragroup differences were statistically insignificant. **Conclusion:** CPP-ACP, chlorhexidine fluoride mouthwash did not adversely affect SBS of orthodontic brackets irrespective of the method of conditioning. Brackets bonded with conventional technique showed greater bond strengths as compared to those bonded with SEP.

Key words: Bonding, casein phosphopeptide amorphous calcium-phosphate, shear bond strength

INTRODUCTION

The bonding of orthodontic attachments has become an integral part of orthodontics.

The bonding of various adhesives to enamel and dentin has developed leaps and bounds over the past 50 years in all areas of dentistry. Buonocore, initially demonstrated the adhesions of acrylic filling materials to enamel, following acid etching with phosphoric acid.[1] It was Newman in 1965, who suggested that this technique can be applied for orthodontic bonding.[2]

There are different adhesive systems for bonding, mainly the conventional etching and self-etching systems. Conventional adhesive systems comprise an enamel

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conditioner, a primer solution and an adhesive resin. When using conventional bonding, the acid etching brings about dissolution of enamel crystals in the prism structure, which produces a permeable enamel surface layer which ranges in depth from 5 to 50 μ.[3] The irregular enamel surface formed helps in micromechanical retention. The amount of dissolution of enamel surface depends upon the type of acid and its concentration.

In self-etching primers (SEPs), the methacrylate group and phosphoric acid ester are combined into a molecule that helps in etching and priming at the same time during the process of bonding. The main advantage of SEP is that two steps are combined and made into a single step. Etching and priming are simultaneously done which helps in eliminating the unwanted effects of unfiltered resin which brings about demineralization.[4]

Loss of surface enamel notably in the gingival third of the crown is being increasingly seen in patients undergoing fixed orthodontic therapy. These enamel decalcifications are also known as white spot lesions (WSLs) due to change in the refractive index of enamel leading to scattering of light and a chalky white appearance.

Several studies have shown that WSLs have a high incidence rate varying from 75.6% in Indians[5] to 96% in Europeans[6] undergoing orthodontic therapy. Several methods have been suggested to counteract the development and progression of WSL ranging from complete appliance removal to topical application of fluoride and recently used titanium oxide coated stainless steel brackets. The use of casein phosphopeptide amorphous calcium-phosphate (CPP-ACP) a milk protein derivative has shown favorable results in decreasing WSLs. Maintenance of oral hygiene with the use of fluoridated toothpaste, mouthwash, etc., has also been recommended as the method to prevent WSLs. However, the presence of fluoride in these products undermines the bond strength of orthodontic brackets by interfering with the binding mechanisms of resins by the formation of fluorapatites suggested by some studies.[7] Hence, it is important to determine the effect of CPP-ACP and fluoridated chlorhexidine (CHX) mouthwash on the shear bond strength (SBS) of orthodontic brackets.

The objectives of the study were:
1. To investigate the effect of fluoride containing CPP-ACP complex on the SBS of brackets bonded with SEP
2. To investigate the effect of fluoride containing CPP-ACP complex on the SBS of brackets bonded with conventional etching primer
3. To investigate the effect of CHX + fluoride mouthwash on the SBS of brackets bonded with SEP
4. To investigate the effect of CHX + fluoride mouthwash on the SBS of brackets bonded with conventional acid etching primer.

MATERIALS AND METHODS

Sixty extracted healthy human premolar teeth with intact buccal enamel, extracted for orthodontic therapy were included in the study. Teeth with caries or any other morphological abnormality were excluded. The teeth were then stored in distilled water. The teeth were mounted vertically group wise using self-cure acrylic, such that the crowns were exposed. The buccal surfaces of the teeth were cleaned and polished with a rubber cup and slurry with nonfluoridated pumice and water, followed by rinsing with a water spray and drying with compressed air.

The sixty extracted teeth were divided into two groups. The first group (Group 1) consisted of 30 teeth bonded with SEP (Transbond Plus, 3M Unitek, Monrovia, CA, USA). The group was further divided into equal subgroups consisted of 10 teeth each. The three subgroups were an untreated control Group (1A), teeth pretreated with fluoridated CPP-ACP paste (MI Paste Plus, GC America, Alsip, Il.) to which brackets were immediately bonded (1B) and teeth pretreated with CHX and fluoride mouthwash to which brackets were bonded 7 days after application (Chlohex-plus, Dr. Reddy's Laboratories, Hyderabad, India) (1C).

The second group (Group 2) consisted of 30 teeth bonded with conventional etching system (35% phosphoric acid and Transbond XT primer, 3M Unitek, Monrovia, CA, USA). It was further divided into subgroups similar to the first group (Group 2A, 2B and 2C, respectively) [Figure 1].

The premolar stainless steel brackets (0.022 slot, MBT prescription, Ormco, Orange, CA, USA) were then bonded to the buccal surfaces of these teeth using a light cured adhesive (Transbond XT, 3M Unitek, Monrovia, CA, USA). The SBS was then measured using a universal testing machine (Model no. 3366, Instron, Norwood, MA, USA).

All data was summarized by mean and standard deviation values. One-factor analysis of variance (ANOVA) was used to compare conventionally and SEP. Multiple comparisons were also performed with Tukey's test when ANOVA yielded significant results indicating that there was difference between the primers.

RESULTS

Tables 1 and 2 show the bond strength of individual groups and the comparison of the six subgroups between the groups and within the groups using ANOVA test. In Group 1, self-etching group, Group 1A had mean SBS of 9.90 ± 2.29 MPa. Group 1B had a mean SBS of 9.83 ± 4.83 MPa compared to Group 1C with a mean SBS of 8.22 ± 2.12 MPa [Figure 2].

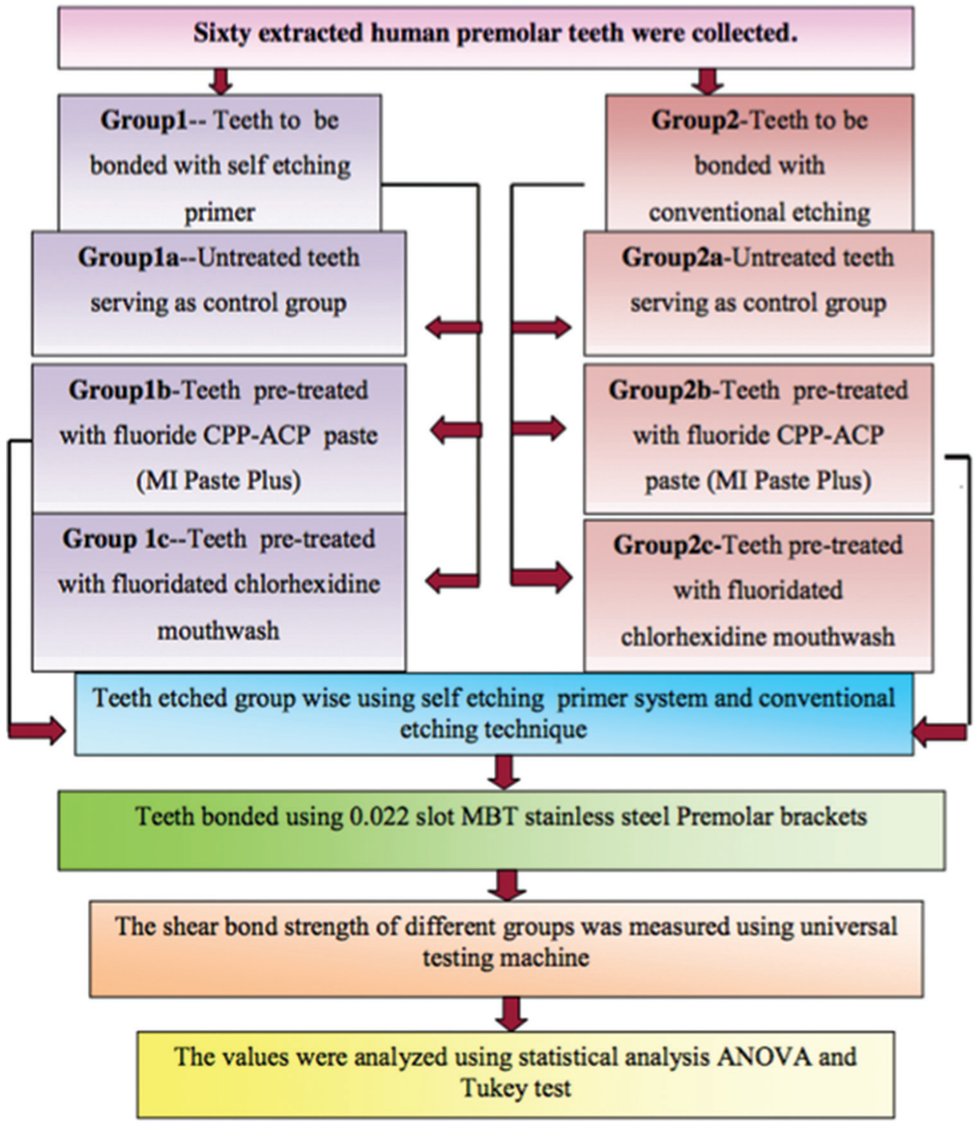

Figure1: Investigation design

Table 1: Shear bond strengths of self-etching primer group (Group 1)

Sub-group	No. of specimens	Minimum*	Maximum*	Mean*	SD*
1 A	10	5.74	12.66	9.9036	2.29466
1 B	10	2.76	20.98	9.8303	4.83438
1 C	10	4.31	10.89	8.2228	2.12168

*Value in Mega Pascals (Mpa). SD=Standard Deviation

Table 2: Shear bond strengths of conventional group (Group 2)

Sub-group	No. of specimens	Minimum*	Maximum*	Mean*	SD*
2 A	10	4.31	10.89	8.2228	2.12168
2 B	10	4.54	21.81	12.4971	5.88858
2 C	10	6.47	16.50	11.4852	3.58934

*Value in Mega Pascals (Mpa). SD=Standard Deviation

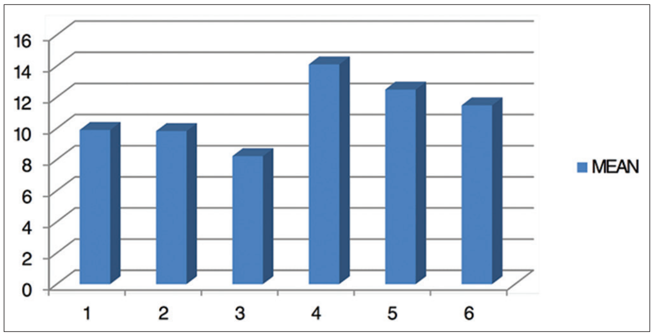

Figure 2: Graphic representation of mean shear bond strength of all the six groups

In Group 2, the conventional etching group, Group 2A had a mean SBS of 14.10 ± 5.56 MPa. In Group 2B teeth the mean bond SBS was 12.49 ± 5.88 MPa, whereas in Group 2C the mean SBS was 11.48 ± 3.58 MPa [Figure 2]. Between the two main groups, the P value was statistically significant ($P > 0.05$) showing that the self-etching group had a lesser bond strength than conventional acid-etch group [Tables 3 and 4].

DISCUSSION

The present study compared the SBS of orthodontic brackets bonded to teeth using SEP and conventional techniques which

Table 3: Inter-group comparisons using analysis of variance

ANOVA					
Bond strength	**Sum of squares**	**df**	**Mean square**	**F**	**Significant**
Inter group	224.297	5	44.859	2.410	0.048*
Intra groups	1005.003	54	18.611		
Total	1229.300	59			

*The mean difference is significant at the 0.05 level

Table 4: Tukey honest significant difference test for multiple comparisons

Multiple comparisons						
Shear bond strength Tukey HSD			SE	Significant	95% CI	
Material (I)	**Material (J)**	**Mean difference (I-J)**			**Lower bound**	**Upper bound**
1a	1b	1.08500	28.55377	1.000	−83.2765	85.4465
	1c	24.87600	28.55377	0.952	−59.4855	109.2375
	2a	−62.25000	28.55377	0.264	−146.6115	22.1115
	2b	−38.38400	28.55377	0.759	−122.7455	45.9775
	2c	−23.40800	28.55377	0.963	−107.7695	60.9535
1b	1a	−1.08500	28.55377	1.000	−85.4465	83.2765
	1c	23.79100	28.55377	0.960	−60.5705	108.1525
	2a	−63.33500	28.55377	0.247	−147.6965	21.0265
	2b	−39.46900	28.55377	0.737	−123.8305	44.8925
	2c	−24.49300	28.55377	0.955	−108.8545	59.8685
1c	1a	−24.87600	28.55377	0.952	−109.2375	59.4855
	1b	−23.79100	28.55377	0.960	−108.1525	60.5705
	2a	−87.12600*	28.55377	0.039*	−171.4875	−2.7645
	2b	−63.26000	28.55377	0.248	−147.6215	21.1015
	2c	−48.28400	28.55377	0.544	−132.6455	36.0775
2a	1a	62.25000	28.55377	0.264	−22.1115	146.6115
	1b	63.33500	28.55377	0.247	−21.0265	147.6965
	1c	87.12600*	28.55377	0.039*	2.7645	171.4875
	2b	23.86600	28.55377	0.959	−60.4955	108.2275
	2c	38.84200	28.55377	0.750	−45.5195	123.2035
2b	1a	38.38400	28.55377	0.759	−45.9775	122.7455
	1b	39.46900	28.55377	0.737	−44.8925	123.8305
	1c	63.26000	28.55377	0.248	−21.1015	147.6215
	2a	−23.86600	28.55377	0.959	−108.2275	60.4955
	2c	14.97600	28.55377	0.995	−69.3855	99.3375
2c	1a	23.40800	28.55377	0.963	−60.9535	107.7695
	1b	24.49300	28.55377	0.955	−59.8685	108.8545
	1c	48.28400	28.55377	0.544	−36.0775	132.6455
	2a	−38.84200	28.55377	0.750	−123.2035	45.5195
	2b	−14.97600	28.55377	0.995	−99.3375	69.3855

*The mean difference is significant at the 0.05 level. HSD – Honest significant difference; SE – Standard error; CI – Confidence interval were pretreated with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) or a chlorhexidine and fluoride mouthwash.

Introduction of bonding revolutionized dentistry in general and orthodontics in particular. It has helped to improve treatment, increase patient comfort, and at the same time eliminate the ill-effects usually associated with banding of teeth. The introduction of SEP in the 1990s further reduced chair side time. However, there has always been disagreement regarding its bond strength as compared to conventional primers. It has been suggested that the bond strength attained using SEP was inferior. There is a large body of evidence supporting as well as refuting the claim.

Previous studies concluded[8-10] that the conventional primer consistently showed better bond strength than SEP.

However, Moule *et al*.[11] reported contrasting findings suggesting that SEP had greater bond strengths than conventional primers.

Bishara *et al*.[12] as well as Buyukyilmaz *et al*.[13] reported that that SBSs of both the systems were not significantly different.

Similarly, Ireland *et al*.[14] from their investigations concluded that there was weak evidence to suggest that bond failures with an SEP were higher than those with conventional etching and priming. They suggested the increased likelihood of bond failure had to be weighed against the time advantage of the SEP when used at the initial bonding appointment.

However, the current study found that there was a statistically significant difference between the two groups. It was observed that the conventional primer group consistently had greater SBS than the SEP group. This included the subgroups where CPP-ACP and CHX with fluoride were used, respectively. These differences can be attributed to different mechanisms of action of the two systems and not the presence of CPP-ACP or CHX.

WSLs are a commonly seen sequela of orthodontic treatment. A number of methods have been suggested to prevent this. These range from topical fluoride applications, dentifrices, mouthwashes, etc.

CPP-ACP is a milk-derived product, a more recent introduction to tackle WSLs. It is claimed that CPP-ACP remineralizes teeth and help prevent caries. Casein phosphopeptides derived from the major milk protein have the ability to stabilize calcium, phosphate and fluoride ions as water-soluble amorphous complexes. These complexes remineralize early stages of tooth decay by replacing calcium and phosphate ions lost due to decay.[15] Presently, CPP-ACP can be administered via sugar-free gum, medicated tooth mousse and fortified dairy milk.

Current literature has contrasting reports of the effect of CPP-ACP on SBS of orthodontic brackets. A number of previous investigations[16-18] have mentioned that CPP-ACP had no detrimental effects on the SBS of orthodontic brackets. However, Dunn[19] reported that ACP-containing composite material failed at significantly lower forces than brackets bonded to teeth with the conventional resin-based composite orthodontic cement. On the other, Adebayo *et al*.[20,21] reported that use of SEP along with CPP-ACP enhanced SBS.

Investigators of the current study did not observe any significant differences in the between the CPP-ACP subgroups (Groups 1b and 2b) and other subgroups in either the conventional or SEP groups.

Hence, on the basis of these findings and previous investigations,[16-18] it can be safely concluded that CPP-ACP did not adversely affect the SBS of brackets bonded with either conventional or SEP.

Chlorhexidine is often used as an active ingredient in commonly prescribed mouthwashes designed to reduce dental plaque and oral bacteria. It actions and efficacy is well documented.[22] However, its role in preventing tooth decay is controversial as clinical data has not been convincing.[23] In addition, there are no clear evidence of its effects on SBS of orthodontic brackets.

Cacciafesta *et al*.[24] assessed the effect of chlorhexidine application on the SBS and concluded that chlorhexidine application immediately before bonding significantly lowered the bond strength values of resin-modified glass ionomer cement (RM-GIC) but did not affect its bond strength when applied 1 week before bonding.

The teeth (Groups 1c and 2c) in the current study were also bonded 1 week after CHX application. It was observed that there were no significant differences between these sub-groups and other sub-groups in either the conventional or SEP groups.

The present study differed from the previously mentioned study in two ways. Firstly, this study used extracted human teeth as opposed to bovine teeth. Second, unlike RM-GIC used in the previous study, the investigators used a light cured composite adhesive. Light cured adhesives have been proven to be more amenable to orthodontic bonding and are also used more widely. These two factors enabled the investigators of this study to provide a more accurate picture of real-time clinical scenarios.

In addition to CHX, additional fluoride supplements are routinely prescribed during orthodontic therapy. Hence, it is also important to consider the effect of fluoride in addition to the CHX mouthwash on SBS of brackets. Previous investigations, for the most part, have studied these two separately and have not considered their effects when acting simultaneously.

The novelty of the current study was that the CHX mouthwash used had incorporated fluoride. Thus, the effects of CHX and fluoride on brackets acting simultaneously on the SBS of orthodontic brackets could be studied.

According to the results, it can be safely concluded that bonding of brackets 1-week post-CHX application did not adversely affect the bracket strength. In addition, the current study concluded that the concurrent use of CHX mouthwash with incorporated with fluoride or other fluoride supplements did not have a detrimental effect on SBS of orthodontic brackets regardless of the method of etching.

CONCLUSION

From the findings of the current study it can be concluded that:

1. The fluoride containing CPP-ACP had no adverse effect on the SBS of brackets bonded with SEP
2. The fluoride containing CPP-ACP had no adverse effect on the SBS of brackets bonded with conventional primers
3. CHX + fluoride mouthwash had no adverse effects on the SBS of brackets bonded with SEP
4. CHX + fluoride mouthwash had no adverse effects on the SBS of brackets bonded with conventional primer
5. Higher SBS was consistently observed in brackets bonded using a conventional primer as compared to SEP.

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Conflicts of interest

There are no conflicts of interest.

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