

Effect of CPP-ACP and CPP-ACP with Fluoride on the Shear Bond Strength of Orthodontic Brackets Bonded with Two Different Adhesive Systems: An In Vitro Study

Authors:

Dr. Shreya Singh¹, Dr. Deepankar Bhatnagar², Dr. Vivek kendre³, Dr. Chaitanya M.S⁴, Dr. Smruti Simron Mohapatra⁵, Dr. Hrishikesh Iyer⁶

^{1,3,4,5,6}Post Graduate Resident, Department of Orthodontics and Dentofacial Orthopedics, Maharishi Markandeshwar College of Dental Science and Research Centre, Mullana, Haryana.

²Head of the Department, Department of Orthodontics and Dentofacial Orthopedics, Maharishi Markandeshwar College of Dental Science and Research Centre, Mullana, Haryana.

Corresponding Author:

Dr. Shreya Singh, Post Graduate Resident, Department of Orthodontics and Dentofacial Orthopedics, Maharishi Markandeshwar College of Dental Science and Research Centre, Mullana, Haryana.

DOI: <https://doi.org/10.5281/zenodo.22318288>

Article Received: 02 August 2026, Accepted: 27 August 2026, Online First: 10 September 2026

ABSTRACT

Introduction: White spot lesions are a frequent problem during fixed orthodontic treatment, mainly because plaque tends to accumulate around brackets and makes oral hygiene difficult. CPP-ACP and CPP-ACP with fluoride (CPP-ACPF) are commonly used to promote enamel remineralization and reduce mineral loss. However, their use before bracket bonding may alter the enamel surface and could influence both bond strength and colour characteristics. **Aim:** To evaluate the effect of CPP-ACP and CPP-ACPF pre-treatment on the shear bond strength of orthodontic brackets bonded with light-cure and chemical-cure adhesive systems and to compare their colour characteristics. **Materials and Methods:** Ninety extracted human premolars were randomly divided into three groups of 30 teeth each: CPP-ACP, CPP-ACPF, and control. Each group was further divided into light-cure and chemical-cure subgroups (n = 15). After standardised enamel pre-treatment and bracket bonding, shear bond strength was measured using a universal testing machine at a crosshead speed of 4 mm/min. Colour parameters were recorded using a spectrophotometer. Statistical analysis was performed using one-way ANOVA, Tukey HSD post-hoc test, and independent samples t-test. **Results:** The control group showed the highest mean shear bond strength with both adhesive systems, followed by CPP-ACPF and CPP-ACP. However, these differences were not statistically significant (p>0.05). Light-cure adhesives showed higher mean bond strength than chemical-cure adhesives, although the difference was also not significant. Colour assessment showed more consistent colour values with light curing, while chemical-cure groups showed greater variation, particularly towards yellow discolouration. **Conclusion:** CPP-ACP and CPP-ACPF pre-treatment did not significantly affect the shear bond strength of orthodontic brackets. CPP-ACPF showed slightly better mean bond strength than CPP-ACP, while light-cure adhesives showed more favourable and consistent colour characteristics. These findings suggest that both remineralizing agents can be used before orthodontic bonding without significantly compromising bond strength. Further clinical studies are needed to confirm these findings under actual oral conditions.

Keywords: CPP-ACP, CPP-ACPF, shear bond strength, orthodontic brackets, light-cure adhesive, chemical-cure adhesive, colour characteristics

INTRODUCTION

Fixed orthodontic treatment is widely used to correct malocclusion and improve dental aesthetics and function. However, brackets and arch wires create additional plaque-retentive areas and make oral hygiene more difficult. This increases the risk of enamel demineralization and the development of white

spot lesions (WSLs), which may compromise the aesthetic outcome of orthodontic treatment [1]. White spot lesions develop when the normal balance between demineralization and remineralization is disturbed. Acids produced by plaque bacteria cause mineral loss from enamel, particularly around orthodontic brackets where plaque removal is difficult. Although fluoride-

containing dentifrices and mouth rinses can reduce this process, they may not completely prevent enamel demineralization [2]. Clinically, these lesions appear as dull, chalky-white areas due to subsurface mineral loss and increased enamel porosity. If the process continues, demineralization may extend deeper into the enamel and eventually involve dentine [3].

The prevention and management of these lesions are therefore important during orthodontic treatment. In addition to fluoride, casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) has gained considerable attention as a remineralizing agent. Casein phosphopeptides can stabilize calcium and phosphate ions and maintain them in a bioavailable form [3,4] CPP-ACP creates a calcium- and phosphate-rich environment around the enamel surface, thereby promoting remineralization and reducing further mineral loss. Studies have shown its potential in improving mineral content and managing early white spot lesions.

The addition of fluoride to CPP-ACP may further improve its remineralizing effect. In CPP-ACP with fluoride (CPP-ACPF), calcium, phosphate, and fluoride ions are maintained in a stabilized form and made available at the enamel surface [4]. This combination may enhance remineralization and increase enamel resistance to acid dissolution. CPP-ACP-based products, such as Tooth Mousse™ and MI Paste™, have therefore been used for the prevention and management of early enamel demineralization [6]. While these agents are beneficial for enamel remineralization, their effect on orthodontic bonding is also important. Adequate shear bond strength (SBS) is necessary to keep brackets attached throughout treatment. Bond strength can be influenced by several factors, including the condition of enamel, etching technique, bracket type, adhesive system, and any surface treatment performed before bonding [7]. Since CPP-ACP and CPP-ACPF interact with the enamel surface, their use before bonding may influence the attachment of orthodontic brackets. Previous studies have reported varying findings. Some have shown an improvement in shear bond strength following CPP-ACP application, whereas others found little or no significant effect [8-15]. The type of adhesive system used may also influence the final bond strength because different adhesives interact differently with the treated enamel surface [16].

It is therefore clinically important that remineralizing agents used to prevent white spot lesions do not interfere with bracket bonding. Although CPP-ACP and CPP-ACPF have shown promising remineralizing effects, their influence on orthodontic bond strength with different adhesive systems requires further evaluation [17-20].

AIM AND OBJECTIVES

AIM

1. To Effect of CPP-ACP and CPP-ACP with Fluoride on the Shear Bond Strength of Orthodontic Brackets Bonded with Two Different Adhesive Systems.

OBJECTIVES

1. To evaluate the shear bond strength of orthodontic brackets bonded to premolars pre-treated with CPP-ACP using a light-cure adhesive system.
2. To evaluate the shear bond strength of orthodontic brackets bonded to premolars pre-treated with CPP-ACP using a chemical-cure adhesive system.
3. To evaluate the shear bond strength of orthodontic brackets bonded to premolars pre-treated with CPP-ACP with fluoride (CPP-ACPF) using a light-cure adhesive system.
4. To evaluate the shear bond strength of orthodontic brackets bonded to premolars pre-treated with CPP-ACP with fluoride (CPP-ACPF) using a chemical-cure adhesive system.
5. To compare the shear bond strength of brackets bonded to untreated control teeth with those pre-treated with CPP-ACP and CPP-ACPF using the two adhesive systems.

MATERIALS AND METHODOLOGY

The present in-vitro study was conducted in the Postgraduate Department of Orthodontics and Dentofacial Orthopedics, Maharishi Markandeshwar College of Dental Sciences and Research (MMCDSR), Mullana, Haryana.

STUDY DESIGN

The study was designed as a laboratory-based in-vitro experimental study to evaluate and compare the effect of CPP-ACP and CPP-ACP with fluoride (CPP-ACPF) pretreatment on the shear bond strength of orthodontic brackets bonded with two different adhesive systems.

SAMPLE SELECTION AND PREPARATION

A total of 90 freshly extracted human premolars were collected from the Department of Oral and Maxillofacial Surgery, Maharishi Markandeshwar College of Dental Sciences and Research, Haryana. The teeth had been extracted as part of the patients' orthodontic treatment plans after obtaining informed consent. Following extraction, the teeth were stored in formaldehyde solution until further preparation.

Inclusion Criteria

- Premolars free from cracks and dental caries
- Absence of hypocalcification and fluorosis
- Teeth without restorations
- No previous history of orthodontic treatment
- Teeth without fractures
- Teeth without visible stains
- Absence of developmental enamel defects

- Absence of tooth jewellery
- Absence of attrition

Exclusion Criteria

- Grossly decayed teeth
- Teeth with cracks or caries
- Teeth with enamel erosion or fluorosis
- Hypocalcified teeth
- Restored teeth
- Teeth with a previous history of orthodontic treatment
- Teeth with fractures or stains
- Teeth with developmental defects
- Presence of tooth jewellery
- Presence of attrition

Each tooth was embedded in Type III dental stone. Dental stone was mixed to the required consistency and transferred into the mould. The teeth were positioned with the roots embedded in the stone while keeping the crowns and buccal surfaces adequately exposed for bracket bonding. The stone was allowed to set for approximately 15 minutes before the blocks were removed from the mould.

STUDY GROUPS

The 90 premolars were randomly divided into three main groups of 30 teeth each according to the type of enamel pretreatment:

Group A-CPP-ACP Group (n = 30): The teeth were pretreated with CPP-ACP in artificial saliva.

Group B-CPP-ACPF Group (n = 30): The teeth were pretreated with CPP-ACP containing fluoride in artificial saliva.

Group C- Control Group (n = 30): The teeth received no CPP-ACP or CPP-ACPF pretreatment and were exposed only to artificial saliva.

Each main group was further divided into two subgroups of 15 teeth according to the adhesive system used:

Group A1 (n = 15): CPP-ACP pretreatment + light-cure adhesive

Group A2 (n = 15): CPP-ACP pretreatment + chemical-cure adhesive

Group B1 (n = 15): CPP-ACPF pretreatment + light-cure adhesive

Group B2 (n = 15): CPP-ACPF pretreatment + chemical-cure adhesive

Group C1 (n = 15): Control + light-cure adhesive

Group C2 (n = 15): Control + chemical-cure adhesive

CROWN SURFACE PRETREATMENT

For Group A, 11.42 g of CPP-ACP tooth mousse was mixed with 100 mL of artificial saliva. Thirty teeth were immersed in this solution for 1 hour per day for 5

consecutive days. Between treatment cycles, the specimens were stored in distilled water.

For Group B, 11.42 g of CPP-ACPF tooth mousse was mixed with 100 mL of artificial saliva. Thirty teeth were immersed in the solution for 1 hour per day for 5 consecutive days and stored in distilled water between treatment cycles. For the control group (Group C), 30 teeth were immersed only in artificial saliva for 1 hour per day for 5 consecutive days and stored in distilled water between cycles.

Following pretreatment, the buccal surfaces of all specimens were cleaned with non-fluoridated pumice before bracket bonding.

Etching and Conditioning

The buccal enamel surfaces of all teeth were etched with 37% phosphoric acid for 30 seconds, rinsed with water for 10 seconds, and dried until a characteristic frosted appearance was obtained. The specimens were then bonded according to their assigned light-cure or chemical-cure adhesive subgroup.

Bracket Bonding

Light-Cure Adhesive Groups

For Groups A1, B1, and C1, a thin and uniform layer of Ortho Solo sealant was applied to the etched and dried enamel surface using a micro brush applicator. Enlight adhesive paste was applied to the bracket base, ensuring complete coverage.

The bracket was positioned on the buccal surface and firmly pressed into place. Excess adhesive around the bracket was carefully removed with an explorer. The adhesive was then polymerized using a light-curing unit for 10 seconds.

Chemical-Cure Adhesive Groups

For Groups A2, B2, and C2, a thin layer of primer was applied to the etched and dried enamel surface as well as to the bracket base. A small amount of chemical-cure adhesive paste was applied to the bracket base, after which the bracket was positioned and firmly pressed against the tooth surface.

Excess adhesive was removed with an explorer, and the bracket was maintained in position for 25 seconds.

Determination of Shear Bond Strength

Shear bond strength was evaluated using a Universal Testing Machine (ASIAN Microdata Acquisition System, MAS-14, Ghaziabad, India) at the Department of Oral Pathology, ITS Dental College, Ghaziabad.

Each dental stone block containing the bonded tooth was securely positioned on the holding platform of the testing machine. A jig attached to the upper grip and

connected to the load cell was positioned so that its pin contacted the bracket at the bracket–tooth interface.

A shear force was applied in an occluso-gingival direction at a crosshead speed of 4 mm/min until bracket failure occurred. The maximum force required to debond each bracket was recorded in Newtons (N).

Shear bond strength was then calculated in megapascals (MPa) using the following formula:

$$\text{Shear Bond Strength (MPa)} = \text{Force required for debonding (N)} / \text{Bracket base area (mm}^2\text{)}$$

The bracket base area used for calculation was 9.03 mm². The obtained shear bond strength values were recorded for all six groups and subsequently subjected to statistical analysis to determine the effect of CPP-ACP and CPP-ACPF pretreatment and the type of adhesive system on orthodontic bracket bond strength.

RESULTS

Comparison of Colour Parameters and Colour Stability Between Light-Cure and Chemical-Cure Adhesive Systems Across the Study Groups

Table 1: Comparison of Colour Parameters and Colour Stability Between Light-Cure and Chemical-Cure Adhesive Systems Across the Study Groups

Group	Parameter	t-value	Mean Difference (L–C)	p-value	95% CI
Group 1	Z	2.320	6.789	0.028	0.795 to 12.783
Group 2	b*	-4.798	-3.976	<0.001	-5.674 to -2.279
Group 2	R_Z	2.320	5.742	0.028	0.672 to 10.812
Group 3	X	2.257	5.862	0.032	0.541 to 11.184
Group 3	Y	2.166	6.009	0.039	0.326 to 11.691
Group 3	Z	2.829	8.801	0.009	2.427 to 15.175
Group 3	b*	-3.668	-3.246	0.001	-5.058 to -1.433
Group 3	R_X	2.092	5.608	0.046	0.117 to 11.099
Group 3	R_Y	2.166	6.009	0.039	0.326 to 11.691
Group 3	R_Z	2.829	7.444	0.009	2.053 to 12.835

The light-cure groups generally maintained better brightness than the chemical-cure groups. In Group 1, both curing methods showed almost similar X, Y, Z, and L* values, suggesting only a small difference in colour. However, the chemical-cure group showed a slightly higher b* value, indicating a greater tendency towards yellow discolouration. The difference was more noticeable in Groups 2 and 3. The light-cure groups showed higher brightness values, whereas the chemical-cure groups showed a greater yellow colour shift. In contrast, the a* values were quite similar between the two curing methods, suggesting little change in the red–green colour range (table 1). Overall, the light-cure adhesive showed better colour stability, particularly in maintaining brightness and limiting yellow discolouration. The difference between the two adhesive systems was small initially but became more apparent across the groups.

Table 2: Comparison of L and C Conditions Across Groups Using Independent Samples t-Test.

Group	Parameter	t-value	Mean Difference (L–C)	p-value	95% CI
Group 1	Z	2.320	6.789	0.028	0.795 to 12.783
Group 2	b*	-4.798	-3.976	<0.001	-5.674 to -2.279
Group 2	R_Z	2.320	5.742	0.028	0.672 to 10.812
Group 2	X	2.257	5.862	0.032	0.541 to 11.184

oup 3		257		032	11.184
oup 3	Gr	Y	2.	6.009	0. 0.326 to
oup 3	Gr	Z	2.	8.801	0. 2.427 to
oup 3	Gr	b*	-	-3.246	0. -5.058 to -
oup 3	Gr	R_X	2.	5.608	0. 0.117 to
oup 3	Gr	R_Y	2.	6.009	0. 0.326 to
oup 3	Gr	R_Z	2.	7.444	0. 2.053 to

The independent samples t-test showed that the difference between the light-cure and chemical-cure groups became more noticeable from Group 1 to Group 3. In Group 1, none of the colour parameters showed a statistically significant difference ($p > 0.05$), suggesting that both curing methods produced similar colour outcomes. In Group 2, significant differences were observed in Z, R_Z, and b*, indicating some variation in colour, particularly along the blue–yellow range. The differences were more evident in Group 3, where X, Y, Z, b*, R_X, R_Y, and R_Z differed significantly between the two curing methods (table 2). Overall, the light-cure group tended to maintain better brightness, whereas the chemical-cure group showed a greater shift towards yellow. These findings suggest that the difference between the two adhesive systems was minimal in Group 1 but became more apparent in Groups 2 and 3.

Descriptive Analysis of Colour Stability of Light-Cure and Chemical-Cure Adhesive Systems

Table 3: Descriptive Analysis of Colour Stability of Light-Cure and Chemical-Cure Adhesive Systems

Para meter	Group 1 Mean	Group 2 Mean	Group 3 Mean	Total Mean
X	48.19	48.66	51.05	49.30
Y	51.72	51.91	54.41	52.68
Z	48.59	47.36	50.87	48.94
L	76.93	76.98	78.44	77.45
a	-2.38	-1.45	-1.40	-1.74
b	7.15	8.63	7.57	7.78
RX	51.16	52.02	54.32	52.50
RY	51.72	51.91	54.41	52.68
RZ	41.10	40.06	43.03	41.40

The descriptive analysis showed a fairly consistent pattern across the study groups. In the light-cure groups, X, Y, Z, L*, and RGB values generally increased from Group 1 to Group 3, indicating better maintenance of brightness. The b* values showed only small changes, suggesting a limited shift towards yellow. In comparison, the chemical-cure groups generally had lower X, Y, Z, L*, and RGB values, along with higher b* values, indicating reduced brightness and a greater tendency towards yellow discolouration. The a* values remained similar with both curing methods, suggesting little variation along the red–green axis (table 3). Overall, the light-cure groups showed better colour characteristics, while the chemical-cure groups showed a more noticeable yellow colour shift, particularly in the later groups.

One-Way ANOVA Comparison of Colour Parameters Under Light-Cure and Chemical-Cure Conditions Across Groups 1–3

Table 4: One-Way ANOVA Comparison of Colour Parameters Under Light-Cure and Chemical-Cure Conditions Across Groups 1–3

Parameter	Sig. (p-value)
<i>(None meet $p < 0.05$ threshold)</i>	-

The one-way ANOVA showed no statistically significant differences among the light-cure groups for any of the colour parameters ($p > 0.05$), suggesting that the colour values remained fairly consistent across Groups 1–3. In the chemical-cure groups, most parameters also showed no significant differences. However, a significant difference was observed for b* ($F = 5.368$, $p = 0.008$), indicating that the degree of yellow colour shift varied among the groups (table 4).

Overall, the light-cure groups showed more consistent colour characteristics, while the chemical-cure groups demonstrated some variation, particularly in the yellow component.

Table 5: Post-Hoc Analysis of Intergroup Differences in Colour Parameters for Light-Cure and Chemical-Cure Adhesive Systems

Condition	Parameter	Significant Comparison	p-value
Light (L)	b*	Groups 1-2-3 (overall)	0.008

The post-hoc analysis showed no significant differences between the light-cure groups for any of the colour parameters ($p > 0.05$), indicating that the colour values remained relatively consistent across the groups. In the chemical-cure groups, a significant difference was observed only for b^* between Groups 1 and 2 (mean difference = -3.03, $p = 0.006$), suggesting a greater yellow colour shift in Group 2 (table 5). Overall, the light-cure groups showed more uniform colour characteristics, while the chemical-cure groups showed some variation, particularly in the yellow colour component.

Table 6: Descriptive Statistics of Shear Bond Strength Across Groups Under Conditions A and B

Descriptives									
Shear Bond Strength									
A_B		Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	
					Lower Bound	Upper Bound			
Condition A	GR	93.74	52.16	13.12	64.52	122.96	25.10	222.00	
	GR	59.04	37.75	9.72	38.43	79.65	22.45	167.00	
	GR	64.52	31.32	8.01	47.43	81.61	30.56	127.00	
	Total	72.43	43.21	6.43	59.44	85.41	22.40	222.00	
Condition B	GR	69.39	54.83	14.12	39.02	99.76	3.37	166.00	
	GR	41.38	36.26	9.31	21.30	61.46	6.44	159.00	
	GR	60.39	41.07	10.12	37.64	83.14	7.77	135.00	
	Total	57.05	45.21	6.71	43.44	70.65	3.37	166.00	

The descriptive analysis of shear bond strength showed that Group 1 had the highest mean values under both conditions. Under Condition A, Group 1 recorded a mean bond strength of 93.74 ± 52.16 MPa, followed by Group 3 (64.52 ± 31.32 MPa) and Group 2 (59.04 ± 37.75 MPa). A similar pattern was observed under Condition B, where Group 1 showed the highest value (69.39 ± 54.83 MPa), followed by Group 3 (60.39 ± 41.07 MPa) and Group 2 (41.38 ± 36.26 MPa) (table 6). Although considerable variation was observed within each group, as reflected by the relatively large standard deviations, group 1 consistently demonstrated the highest mean shear bond strength under both conditions.

One-Way ANOVA Comparison of Shear Bond Strength Among Groups Under Conditions A and B

Table 7: One-Way ANOVA Comparison of Shear Bond Strength Among Groups Under Conditions A and B

ANOVA						
Shear Bond Strength						
A_B		Sum of Squares	df	Mean Square	F	Sig.
Between		10435.60	2	5217.80	3.0	.05

	Groups	2		1	53	8
	Within Groups	4	71772.50	42	1708.86	
	Total	6	82208.10	44		
	Between Groups	2	6132.060	2	3066.03	1.5
	Within Groups	7	84103.11	42	2002.45	.22
	Total	8	90235.17	44		

The one-way ANOVA showed some variation in shear bond strength among Groups 1, 2, and 3 under Condition A; however, the difference did not reach statistical significance ($F(2,42) = 3.053$, $p = 0.058$). Under Condition B, the differences among the three groups were also not statistically significant ($F(2,42) = 1.531$, $p = 0.228$) (table 7). Overall, greater variation in bond strength was observed under Condition A, while the values under Condition B were comparatively more uniform across the groups.

Tukey HSD Post Hoc Multiple Comparisons for Shear Bond Strength Across Groups (Conditions A and B)

Table 8: Tukey HSD Post Hoc Multiple Comparisons for Shear Bond Strength Across Groups (Conditions A and B)

Multiple Comparisons								
Dependent Variable: Shear Bond Strength								
Tukey HSD								
B	(I) GROUPS	(J) GROUPS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
						Lower Bound	Upper Bound	
A	GR OUP 1	GR OUP 2	34.69467	15.067	.0	-1.9778	71.3671	
		GR OUP 3	29.21200	15.041	.1	-7.4604	65.8844	
		GR OUP 1	-34.69467	15.067	.0	-71.3671	1.9778	
	GR OUP 2	GR OUP 3	-5.48267	15.030	.9	-42.1551	31.1898	
		GR OUP 1	-29.21200	15.041	.1	-65.8844	7.4604	
		GR OUP 2	5.48267	15.030	.9	-31.1898	42.1551	
	GR OUP 3	GR OUP 1	34.69467	15.067	.0	1.9778	71.3671	
		GR OUP 2	29.21200	15.041	.1	-7.4604	65.8844	
		GR OUP 3	-29.21200	15.041	.1	-65.8844	7.4604	
B	GR OUP 1	GR OUP 2	28.00467	16.312	.2	-11.6931	67.7025	
		GR OUP 3	9.00133	16.347	.8	-30.6965	48.6991	
		GR OUP 1	-28.00467	16.312	.2	-67.7025	11.6931	
	GR OUP 2	GR OUP 3	-19.00333	16.382	.4	-58.7011	20.6945	
		GR OUP 1	-9.00133	16.347	.8	-48.6991	30.6965	
		GR OUP 2	19.00333	16.382	.4	-20.6945	58.7011	
	GR OUP 3	GR OUP 1	9.00133	16.347	.8	-30.6965	48.6991	
		GR OUP 2	-9.00133	16.347	.8	-48.6991	30.6965	
		GR OUP 3	-19.00333	16.382	.4	-58.7011	20.6945	

The Tukey HSD post-hoc analysis showed no statistically significant differences in shear bond strength between any of the groups under either condition. Under Condition A, the difference between Groups 1 and 2 was close to

significance (mean difference = 34.69, $p = 0.067$), with Group 1 showing a higher mean bond strength. However, the differences between Groups 1 and 3 ($p = 0.141$) and Groups 2 and 3 ($p = 0.930$) were not significant. Under Condition B, none of the pairwise comparisons showed a significant difference ($p > 0.05$) (table 8). Overall, the findings indicate broadly comparable shear bond strength among the groups, with only a noticeable but non-significant difference between Groups 1 and 2 under Condition A.

Table 9: Independent Samples t-Test Comparison of Shear Bond Strength between Conditions A and B Across Groups 1–3

Group Statistics						
GROUPS		B	N	Mean	Std. Deviation	Std. Error Mean
GROUP 1	Shear Bond Strength	A	15	93.7353	52.15988	13.46762
		B	15	69.3873	54.82669	14.15619
GROUP 2	Shear Bond Strength	A	15	59.0407	37.74713	9.74627
		B	15	41.3827	36.26121	9.36260
GROUP 3	Shear Bond Strength	A	15	64.5233	31.32264	8.08747
		B	15	60.393860	41.06732	10.60354

The descriptive analysis showed that Group 1 had the highest mean shear bond strength under both conditions. Under Condition A, Group 1 recorded a mean value of 93.74 ± 52.16 MPa, followed by Group 3 (64.52 ± 31.32 MPa) and Group 2 (59.04 ± 37.75 MPa). A similar pattern was observed under Condition B, with Group 1 showing the highest mean value (69.39 ± 54.83 MPa), followed by Group 3 (60.39 ± 41.07 MPa) and Group 2 (41.38 ± 36.26 MPa) (table 9). Overall, Group 1 consistently showed higher mean shear bond strength, although considerable variability was observed within the groups.

Independent Samples t-Test Comparison of Shear Bond Strength Between Groups, Including Levene's Test for Equality of Variances

Table 10: Independent Samples t-Test Comparison of Shear Bond Strength Between Groups, Including Levene's Test for Equality of Variances

Independent Samples Test											
GROUPS			Levene's Test for Equality of Variances		t-test for Equality of Means						
			df	Significance	t	df	Significance (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
GROUP 1	Shear Bond Strength	Equal variances assumed								Lower	Upper
			189	.667	.246	8	.223	34800	24.53905	19.567594	64.37194
GROUP 2	Shear Bond Strength	Equal variances assumed									
			191	.666	.307	8	.202	65800	17.51474	13.002568	45.34168

GRO UP 3	hear Bond Stren gth	Equ al variances assumed	.287	266	310	8	759	3733	4.1	13.	2	3
									33575	3.17971	1.45437	

The independent samples t-test showed no statistically significant difference in shear bond strength between Conditions A and B in any of the three groups. In Group 1, the mean difference was 24.35 MPa ($t = 1.246$, $p = 0.223$), while Group 2 showed a mean difference of 17.66 MPa ($t = 1.307$, $p = 0.202$). Group 3 showed the smallest difference of 4.14 MPa ($t = 0.310$, $p = 0.759$). Levene's test was non-significant for all three groups ($p > 0.05$), indicating that the assumption of equal variances was satisfied. Overall, shear bond strength was comparable between Conditions A and B across all three groups (table 10).

DISCUSSION

Successful orthodontic bonding requires adequate bracket retention without compromising enamel health. This is particularly important during fixed orthodontic treatment, where plaque accumulation around brackets increases the risk of enamel demineralization and white spot lesions (WSLs). CPP-ACP and CPP-ACP-F are commonly used remineralizing agents because they provide bioavailable calcium and phosphate, while the addition of fluoride may further improve enamel resistance to demineralization [21,22]. However, as these agents modify the enamel surface, they may also influence resin penetration and bracket bond strength [9].

The present in vitro study evaluated the effect of CPP-ACP and CPP-ACP-F pre-treatment on the shear bond strength (SBS) of orthodontic brackets bonded with light-cure and chemical-cure adhesive systems. This is clinically relevant because remineralizing agents should protect enamel without reducing the strength required to maintain brackets throughout treatment [23,24,25]. Under light-cure conditions, the control group showed the highest mean SBS (93.74 ± 52.16 MPa), followed by CPP-ACP-F (64.52 ± 31.32 MPa) and CPP-ACP (59.04 ± 37.75 MPa). Although these values differed numerically, the overall difference was not statistically significant ($F = 3.053$, $p = 0.058$). This agrees with Naseh *et al.* [12] and Ladhe *et al.* [15], who also reported that CPP-ACP-based pre-treatment did not significantly compromise orthodontic bond strength.

Under chemical-cure conditions, the control group again showed the highest mean SBS (69.39 ± 54.83 MPa), followed by CPP-ACP-F (60.39 ± 41.07 MPa) and CPP-ACP (41.38 ± 36.26 MPa). The differences were again not statistically significant ($F = 1.531$, $p = 0.228$). The lower value observed with CPP-ACP may suggest a possible reduction in bond strength, but this should be interpreted cautiously because of the wide variation within the groups. Similar trends have also been reported by Ladhe *et al.* [15]. When the two adhesive systems were compared, light-cure adhesives showed higher mean SBS than chemical-cure adhesives in all three groups. However, none of these

differences reached statistical significance. The findings therefore suggest a numerical advantage with light curing rather than a statistically proven superiority. This pattern is consistent with previous reports indicating favourable bonding characteristics and better working-time control with light-cure systems [10,17].

Another important finding was that CPP-ACP-F showed slightly higher mean SBS than CPP-ACP under both curing conditions. However, these differences were not statistically significant. The relatively better performance of CPP-ACP-F may be related to the combined availability of calcium, phosphate, and fluoride ions at the enamel surface [6,18,19]. Çehreli *et al.* [26] also reported that fluoride-containing CPP-ACP did not adversely affect orthodontic bracket bond strength. The colour analysis also showed differences between the two curing systems. Light-cured samples generally maintained more uniform brightness, whereas chemical-cure samples showed a greater tendency towards yellow colour change. In the chemical-cure groups, b^* showed a significant intergroup difference ($F = 5.368$, $p = 0.008$), and post-hoc analysis showed a significant difference between Groups 1 and 2 (mean difference = -3.03 , $p = 0.006$). In contrast, colour parameters remained relatively uniform among the light-cure groups. These findings are comparable with those of Aswani *et al.* [21], who reported that resin-treated enamel may undergo colour changes depending on the characteristics of the resin material and environmental exposure. The greater yellow shift seen with chemical curing may be related to differences in polymerization chemistry and initiator systems. However, as the present study assessed laboratory colour parameters rather than long-term clinical discolouration, these results should be interpreted cautiously.

Overall, neither CPP-ACP nor CPP-ACP-F caused a statistically significant reduction in shear bond strength with either adhesive system. The control group showed the highest mean SBS, while CPP-ACP-F showed slightly better values than CPP-ACP. Light-cure adhesives also tended to show higher bond strength and more uniform colour characteristics than chemical-

cure adhesives, although the differences in SBS were not statistically significant. These findings suggest that CPP-ACP and CPP-ACP-F can be used as enamel-protective pre-treatments without significantly compromising orthodontic bracket bonding. CPP-ACP-F may offer an additional remineralizing advantage because of its fluoride content [6,18,19,26] However, larger clinical studies with longer follow-up are needed to confirm these laboratory findings.

Strengths of the Study

The major strengths of this study include the use of a controlled in vitro experimental design with standardized enamel preparation and orthodontic bracket-bonding procedures. The study included both CPP-ACP and CPP-ACPF pretreatment groups along with a control group, allowing direct comparison of their effects on the shear bond strength of orthodontic brackets. Furthermore, two different adhesive curing systems, namely light-cure and chemical-cure adhesives, were evaluated, providing a broader assessment of the influence of pretreatment on bracket bonding. The study also evaluated colour parameters in addition to shear bond strength, thereby providing supplementary information regarding the potential aesthetic effects associated with the evaluated pretreatment and adhesive protocols.

Limitations of the Study

This study has several limitations. First, it was conducted under controlled in vitro conditions and therefore the findings may not fully represent the complex oral environment. Factors such as saliva, mastication, pH fluctuations, temperature changes, plaque accumulation, and intraoral mechanical stresses cannot be completely reproduced under laboratory conditions. Second, the study used extracted premolars, and variations in enamel characteristics among individual teeth may have contributed to the observed variability in shear bond strength. Third, the sample size was limited to 90 specimens, which may have reduced the ability to detect small differences between groups. Fourth, the colour assessment was performed under laboratory conditions and may not reflect long-term clinical colour changes in the oral environment. Finally, the study did not evaluate long-term bracket survival, enamel demineralization, or clinical remineralization outcomes. Therefore, the findings should be interpreted cautiously and further validated through larger and well-designed clinical studies.

Future Research

Future studies should include larger sample sizes and standardized experimental protocols to improve the reliability and generalizability of the findings. Long-term clinical studies are warranted to evaluate bracket survival, enamel demineralization, white spot lesion development, and colour stability under actual oral

conditions. Further research should also investigate different concentrations and application protocols of CPP-ACP and CPP-ACPF, as well as their effects when used with different orthodontic adhesive systems. Incorporation of thermocycling, simulated oral mechanical loading, and prolonged aging protocols may provide a more clinically relevant assessment of bond strength. Comparative clinical studies are also recommended to determine whether the observed laboratory findings translate into meaningful clinical benefits.

CONCLUSION

Within the limitations of this in vitro study, CPP-ACP and CPP-ACP-F did not significantly reduce orthodontic bracket shear bond strength. The control group showed the highest mean bond strength under both curing conditions. CPP-ACP-F showed slightly higher mean bond strength than CPP-ACP, although the difference was not statistically significant. Light-cure adhesives showed higher mean bond strength than chemical-cure adhesives, but without statistical significance. Both adhesive systems therefore demonstrated comparable bonding performance. Light-cured samples showed better maintenance of brightness and more uniform colour characteristics. Chemical-cured samples showed a greater tendency towards yellow discolouration. Overall, CPP-ACP and CPP-ACP-F may be used before orthodontic bonding without significantly affecting bond strength. Further long-term clinical studies with larger samples are recommended to confirm these findings.

Declarations

Ethical Approval

The study was conducted in accordance with the ethical principles applicable to research involving extracted human teeth. The extracted teeth were obtained from patients undergoing orthodontic treatment after informed consent, and the specimens were used exclusively for research purposes after extraction. Ethical considerations regarding the use of extracted human teeth were followed throughout the study.

Informed Consent

The extracted teeth were obtained from patients undergoing orthodontic treatment after obtaining informed consent for extraction and use of the extracted teeth for research purposes, as applicable.

Funding

No external funding was received for this study.

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

All authors contributed to the conception and design of the study, data collection, analysis and interpretation of the results, drafting of the manuscript, and critical revision of the manuscript. All authors read and approved the final version of the manuscript.

Data Availability Statement

The data generated and/or analysed during the present study are available from the corresponding author on reasonable request.

Acknowledgments

The authors would like to acknowledge the Department of Orthodontics and Dentofacial Orthopedics, Maharishi Markandeshwar College of Dental Sciences and Research, Mullana, Haryana, and the Department of Oral Pathology, ITS Dental College, Ghaziabad, for providing the facilities and technical support required for this study.

REFERENCES

1. Al-Khateeb, S., Exterkate, R. A. M., de Josselin de Jong, E., Angmar-Månsson, B., & ten Cate, J. M. (2002). Light-induced fluorescence studies on dehydration of incipient enamel lesions. *Caries Research*, 36(1), 25-33.
2. Andersson, A., Sköld-Larsson, K., Hallgren, A., Petersson, L. G., & Twetman, S. (2007). Effect of a dental cream containing amorphous calcium phosphate complexes on white spot lesion regression assessed by laser fluorescence. *International Journal of Paediatric Dentistry*, 17(6), 429-435.
3. Anttonen, V., Seppä, L., & Hausen, H. (2004). A follow-up study of the use of DIAGNOdent for monitoring fissure caries in children. *Community Dentistry and Oral Epidemiology*, 32(4), 302-307.
4. Aref, N. S., & Alrasheed, M. K. (2022). Casein phosphopeptide amorphous calcium phosphate and universal adhesive resin as a complementary approach for management of white spot lesions: An in-vitro study. *Progress in Orthodontics*, 23(1).
5. Ashritha, M., & Ramani, P. (2021). Prevalence of caries secondary to the orthodontic treatment in patients visiting a private dental hospital—A retrospective study. *Journal of Pharmaceutical Research International*, 386-400.
6. Aswani, R. S., Chandrappa, V., Uloopi, K. S., Chandrasekhar, R., & RojaRamya, K. S. (2020). Resin infiltration of artificial enamel lesions: Evaluation of penetration depth, surface roughness and color stability. *International Journal of Clinical Pediatric Dentistry*, 13(2), 126-130. <https://doi.org/10.5005/jp-journals-10005-1692>
7. Bakhadher, W., Halawany, H., Talic, N., Abraham, N., & Jacob, V. (2015). Factors affecting the shear bond strength of orthodontic brackets: A review of in vitro studies. *Acta Medica (Hradec Kralove)*, 58(2), 43-48.
8. Çehreli, S. B., Şar, C., Polat-Özsoy, Ö., Ünver, B., & Özsoy, S. (2012). Effects of a fluoride-containing casein phosphopeptide-amorphous calcium phosphate complex on the shear bond strength of orthodontic brackets. *European Journal of Orthodontics*, 34(2), 193-197. <https://doi.org/10.1093/ejo/cjq183>
9. Daneshkazemi, P., Sadeghian, S., & Khodaei, M. (2021). Shear bond strength of orthodontic brackets on intact and demineralized enamel after application of resin infiltrant, fluoride varnish and casein phosphopeptide-amorphous calcium phosphate remineralizing agents: In-vitro study. *International Orthodontics*.
10. Farooq, I., Moheet, I. A., Imran, Z., & Farooq, U. (2013). A review of novel dental caries preventive material: Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) complex. *King Saud University Journal of Dental Sciences*, 4(2), 47-51.
11. Gorton, J., & Featherstone, J. D. B. (2003). In vivo inhibition of demineralization around orthodontic brackets. *American Journal of Orthodontics and Dentofacial Orthopedics*, 123(1), 10-14.
12. Indrapriyadharshini, K., Madan Kumar, P., Sharma, K., & Iyer, K. (2018). Remineralizing potential of CPP-ACP in white spot lesions-A systematic review. *Indian Journal of Dental Research*, 29(4), 487.
13. Jayarajan, J., Janardhanam, P., Jayakumar, P., & Deepika. (2011). Efficacy of CPP-ACP and CPP-ACPF on enamel remineralization-An in vitro study using scanning electron microscope and DIAGNOdent®. *Indian Journal of Dental Research*, 22(1), 77.
14. Ladhe, K. A., Sastri, M. R., Madaan, J. B., & Vakil, K. K. (2014). Effect of remineralizing agents on bond strength of orthodontic brackets: An in vitro study. *Progress in Orthodontics*, 15(1).
15. Naseh, R., Fallahzadeh, F., Atai, M., Mortezaei, O., & Setayeshrad, R. (2017). Casein

- phosphopeptide-amorphous calcium phosphate effects on brackets shear bond strength and enamel damage. *Journal of Clinical and Experimental Dentistry*.
16. Ogaard, B., Rølla, G., & Arends, J. (1988). Orthodontic appliances and enamel demineralization. Part 1. Lesion development. *American Journal of Orthodontics and Dentofacial Orthopedics*, 94(1), 68-73.
 17. Øgaard, B., Rølla, G., Arends, J., & ten Cate, J. M. (1988). Orthodontic appliances and enamel demineralization. Part 2. Prevention and treatment of lesions. *American Journal of Orthodontics and Dentofacial Orthopedics*, 94(2), 123-128.
 18. Omidkhoda, M., Eslami, N., Mazloun, M., & Entezari, M. (2023). Evaluation of bond strength of orthodontic brackets using light- and chemical-cure adhesive systems over time: An in-vitro study. *Journal of Orthodontic Science*, 12(1), 6.
 19. Reynolds, E. C. (1997). Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. *Journal of Dental Research*, 76(9), 1587-1595.
 20. Reynolds, E. C. (1998). Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: A review. *Special Care in Dentistry*, 18(1), 8-16.
 21. Reynolds, E. C. (2009). Casein phosphopeptide-amorphous calcium phosphate: The scientific evidence. *Advances in Dental Research*, 21(1), 25-29.
 22. Reynolds, E. C., Cai, F., Cochrane, N. J., Shen, P., Walker, G. D., Morgan, M. V., & Reynolds, C. (2008). Fluoride and casein phosphopeptide-amorphous calcium phosphate. *Journal of Dental Research*, 87(4), 344-348.
 23. Roelofs, T., Merkens, N., Roelofs, J., Bronkhorst, E., & Breuning, H. (2017). A retrospective survey of the causes of bracket- and tube-bonding failures. *The Angle Orthodontist*, 87(1), 51-56.
<https://doi.org/10.2319/030516-166.1>
 24. Srinivasan, N., Kavitha, M., & Loganathan, S. C. (2010). Comparison of the remineralization potential of CPP-ACP and CPP-ACP with 900 ppm fluoride on eroded human enamel: An in situ study. *Archives of Oral Biology*, 55(7), 541-544.
 25. Wu, G., Liu, X., & Hou, Y. (2010). Analysis of the effect of CPP-ACP tooth mousse on enamel remineralization by circularly polarized images. *The Angle Orthodontist*, 80(5), 933-938.
 26. Xiaojun, D., Jing, L., Xuehua, G., Hong, R., Youcheng, Y., Zhangyu, G., et al. (2009). Effects of CPP-ACP paste on the shear bond strength of orthodontic brackets. *The Angle Orthodontist*, 79(5), 945-950.