before and after taking the readings for each patient. Prior to the start of this study, the blinded examiner was introduced to the measurement protocol, and the use of the DIAGNOdent® and its calibration for laser fluorescence. Intra-examiner reliabilities were assessed by selecting 20 surfaces measuring by device in a patient without orthodontic appliances. The same 20 surfaces were remeasured 1 week later and the intraclass correlation coefficient (ICC) was calculated and found to be 0.91. The same device and tip were used for all the readings throughout the study. Each tooth was pumiced with a fluoride-free paste and then dried using the dental unit air water syringe. Then, the DIAGNOdent® tip was placed on the measurement site, slightly tilted with a circular movement on all sides around the bracket until it reached the maximum reading for each side (mesial, distal, incisal, and gingival). The measurements were done at three different time intervals: immediately after bonding (T0), 1 month after bonding (T1), and then 6 months after bonding (T2). For each group, the demineralization variation (ΔD) was determined as the change from baseline demineralization (T0) score to the highest score (T1) or (T2).

Data Analysis

Data entry and analysis were done using the IBM SPSS version 22 for Mac OS.

Results

A two-way analysis of variance was used to test the effect of adhesive materials (ACP and control) and time intervals, base line (T0), 1 month (T1), and 6 months (T2), show that there was a significant interaction between the adhesive materials and the times (p value = 0.000) (Table 1).

Table 1: Interaction between the adhesive materials and times

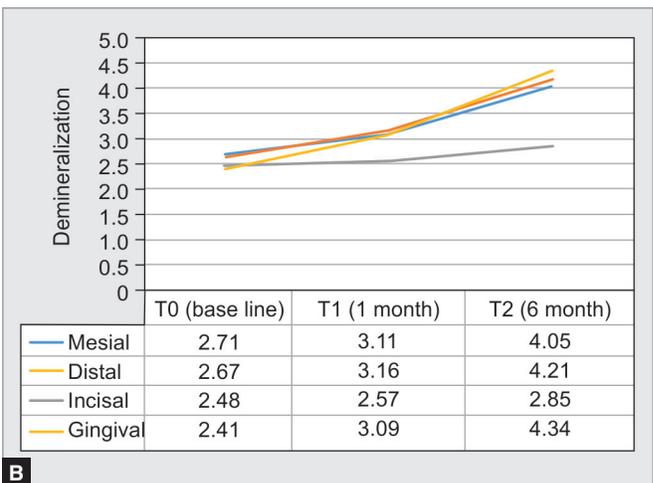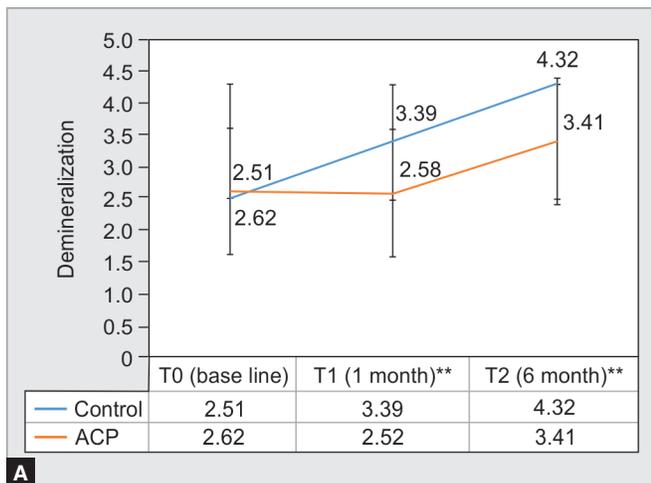| Tests of between-subjects effects			
Dependent variable: demineralization			
Source	Mean square	F	Sig.
Intercept	30137.503	13587.48	0.000*
Group	222.779	100.440	0.000*
Interval	447.900	201.936	0.000*
Group* interval	81.137	36.581	0.000*

**p < 0.01 (highly significant)

Figure 3A shows that there was no significant difference (p = 0.208) between control 2.51 (±1.21) and ACP 2.62 (±1.54) groups at the base line (T0). At 1 month (T1), there was a significant difference between control 3.39 (±1.29) and ACP 2.58 (±1.11) groups (p = 0.000). Furthermore, at six months (T2), there was a significant difference between control 4.32 (±2.32) and ACP 3.41 (±1.11) groups (p = 0.000).

In both control and ACP groups, one-way ANOVA repeated measurements show that there was a significant difference in the demineralization between different time intervals. To further analyze the interaction between different time points, a paired t test was performed and there was a significant difference (p = 0.000) between all three-time intervals within the control group of 6 months (T2) significantly higher than 1 month (T1) and 1 month (T1) was significantly higher than base line (T0). Similarly, for the ACP group, a paired t test show that there was no significant difference between means of demineralization at T0 and T1 (p = 0.321) but the means of demineralization at both base line (T0) and 1 month (T1) were significantly lower than the mean of demineralization at 6 months (T2) (p = 0.000) individually (Tables 2 and 3).

In the control group, the correlation coefficient between the time intervals and demineralization showed a significant positive moderate relationship (p value = 0.000). While in the ACP group, the correlation coefficient between the time intervals and demineralization was r = 0.243, showing a significant positive weak relationship (p value = 0.000) (Table 4).

In Table 5 and Figure 3B, it was observed that at T0 (base line), the one-way ANOVA shows that there was a significant difference between surfaces demineralization (p = 0.041); however, Games–Howell multiple comparison refuses this result and shows that there was no significant difference between surfaces demineralization around the brackets. At 1 month (T1), the one-way ANOVA shows that there was a significant difference between surfaces demineralization (p = 0.00). Furthermore, the Games–Howell multiple comparison shows the incisal surface to have significantly lower deminiralization than other surfaces around the brackets (mesial, distal, and gingival to the brackets) (p = 0.00). At 6 months (T2), there was a significant difference between surfaces demineralization (p = 0.00). The incisal surface to the bracket was significantly lower than other surfaces around the brackets (mesial, distal, and gingival to the brackets) (p = 0.00).

A	T0 (base line)	T1 (1 month)**	T2 (6 month)**
Control	2.51	3.39	4.32
ACP	2.62	2.52	3.41

B	T0 (base line)	T1 (1 month)	T2 (6 month)
Mesial	2.71	3.11	4.05
Distal	2.67	3.16	4.21
Incisal	2.48	2.57	2.85
Gingival	2.41	3.09	4.34

Figs 3A and B: (A) Demineralization between groups in different time intervals (**p = 0.000); (B) The mean surface demineralization around the orthodontic brackets at different time intervals (control or ACP or pooled)

Table 2: Comparison of demineralization within each group at different time intervals by one-way ANOVA repeated measurements' test

Group	Time intervals	Mean	Std. deviation	Sig.
Control	T0 (baseline)	2.51	1.215	0.000*
	T1 (1 month)	3.39	1.288	
	T2 (6 months)	4.32	2.315	
ACP	T0 (baseline)	2.62	1.539	0.000*
	T1 (1 month)	2.58	1.106	
	T2 (6 months)	3.41	1.112	

Table 3: Paired *t* test comparing time intervals within each group

Group	Time intervals	Time intervals	Mean difference	Sig.
Control	T0 (baseline)	T1 (1 month)	−0.880*	0.000*
	T0 (baseline)	T2 (6 months)	−1.809*	0.000*
	T1 (1 month)	T2 (6 months)	−0.929*	0.000*
ACP	T0 (baseline)	T1 (1 month)	0.045	0.321
	T0 (baseline)	T2 (6 months)	−0.787*	0.000*
	T1 (1 month)	T2 (6 months)	−0.832*	0.000*

Table 4: Correlation between time and demineralization within each group

Demineralization in		Time intervals
Control	Pearson correlation	0.402
	Sig. (2-tailed)	0.000**
	N	1524
ACP	Pearson correlation	0.243
	Sig. (2-tailed)	0.000**
	N	1536

Discussion

Demineralization of enamel is an expected side effect associated with fixed orthodontic treatment, especially with poor oral hygiene patients.[15] In the orthodontic database, the role of ACP, the bioactive material, capable of reducing the new increments of dental caries, has been investigated in various studies.[16,17] The present clinical study evaluated the *in vivo* effect of orthodontic ACP containing adhesive compared with the conventional orthodontic adhesive material on enamel demineralization around orthodontic brackets by using the DIAGNOdent device. The added advantage associated with ACP is the disordered structure, making the material exceedingly reactive with body fluids. On the evidence-based hierarchy, it has been found in the literature that osteo-conductivity of ACP was slightly better than hydro apatite when used *in vivo*. External factors like plaque, calculus, stains, and certain other fluorescent materials could be a hindrance to the penetrating ability of laser used by the DIAGNOdent device. To overcome such obstructions, facial surfaces of all the teeth were cleaned using a rotating brush and water jet, which may reduce any false positive results. The subjects were randomly selected and each subject underwent split-mouth design as it eliminates variables pertaining to difference in patient's cooperation and diet. WSLs have the potential of formation within 4 weeks from the beginning of fixed orthodontic treatment.[4] A 6-month measurement was done to evaluate the alteration in effect of the experimental adhesive containing ACP over a long period of time.

The present findings showed that ACP containing adhesive had a preventive effect on enamel demineralization in the four surfaces around the orthodontic brackets. This was in agreement with the study done by Uysal et al.[7] who demonstrated a positive effect of ACP containing adhesive on enamel demineralization

Table 5: Comparison of difference between orthodontic surfaces demineralization at different time intervals

		ANOVA				
		Sum of squares	Df	Mean square	F	Sig.
Baseline	Between groups	15.878	3	5.293	2.763	0.041
	Within groups	1946.588	1016	1.916		
	Total	1962.467	1019			
1 month	Between groups	58.780	3	19.593	12.624	0.000
	Within groups	1576.902	1016	1.552		
	Total	1635.682	1019			
6 months	Between groups	360.957	3	120.319	38.203	0.000
	Within groups	3199.827	1016	3.149		
	Total	3560.784	1019			

Games–Howell multiple comparison between each surface demineralization

Dependent variable	Surface	Surface	Mean difference	Sig.
Baseline (T0)	Mesial	Distal	0.04314	0.985
		Incisal	0.23137	0.320
		Gingival	0.29804	0.054
	Distal	Incisal	0.18824	0.457
		Gingival	0.25490	0.088
	Incisal	Gingival	0.06667	0.947
1 month (T1)	Mesial	Distal	−0.04314	0.982
		Incisal	0.54510*	0.000**
		Gingival	0.02353	0.997
	Distal	Incisal	0.58824*	0.000**
		Gingival	0.06667	0.935
	Incisal	Gingival	−0.52157*	0.000**
6 months (T2)	Mesial	Distal	−0.15686	0.813
		Incisal	1.20784*	0.000**
		Gingival	−0.28235	0.351
	Distal	Incisal	1.36471*	0.000**
		Gingival	−0.12549	0.896
	Incisal	Gingival	−1.49020*	0.000**

after the first 30 days *in vitro* and in other *in vivo* studies.[18] Another study evaluated the potential of ACP containing adhesive, fluoride varnish, resin sealer, and MI Paste under *in vitro* conditions to prevent enamel demineralization around orthodontic brackets, the finding observed a positive, though not significant effect of both ACP containing adhesive and MI Paste.[19]

In all previous studies, the experimental period was just 30 days or less while in the present study, the duration of follow up ended at 6 months which may help evaluate the material performance over an extended period of time. During the follow up for 6 months, the ACP containing adhesive maintained its advantage over the control adhesive but both groups exhibited an increase in enamel demineralization which could be attributed to the initial release of calcium and phosphate ions into the tooth surface within first 30 days. A study done by Richards et al.[20] reported that ACP containing adhesive lose their chemical release potential after 56 days. Currently,

there is no study evaluating the chemical effect of ACP containing adhesive over prolonged periods and whether their ions releasing capacity could replenish by any means.

In this study, all surfaces around orthodontic brackets were evaluated. The gingival, mesial, and distal surfaces around the brackets had the highest enamel demineralization value and they were statistically higher than the incisal surface, this might be due to the difficulty in cleaning of these areas, which was in agreement with the study done by Sukontapatipark et al.[21] It was reported that those sites had the highest rate of WSL formation.[5,22–23] Incorporation of ACP into an orthodontic adhesive material decreases the bacterial adhesion and lesion depth formation better than the resin control.[14] Another advantage of ACP is that it gives higher enamel microhardness than those bonded with conventional composite resin.[13]

The results of the study should be viewed in the light of limitations; first of all, the demineralization of enamel is a continuous process with periods of remineralization and demineralization and the brackets are placed for a 1 year and above, but the study lasted for 6 months only. The oral hygiene practices, cultural factors, dietary patterns, availability of food items, caries activity of the oral cavity, and many more risk factors should be taken into consideration before coming to a conclusion, hence, the results of the present study cannot be generalized to the society. Further multicentric studies from different parts of the world with more multicentric robust sample size and longer follow up periods will be required to give more reliable conclusions. Nevertheless, the present study gives a platform to use ACP as an adhesive for brackets' placements in orthodontics so that the enamel demineralization can be reduced and the prognosis can be further improved. This study also opens the avenue for further research worldwide. Greater strides in the development of tissue engineering technology and techniques and day-to-day enrichment in applied dental material sciences, it is thought that ACP will be used comprehensively in the coming future. It is a promising material, which can stop demineralization and can prevent dental caries due to placement of orthodontic brackets.

CONCLUSIONS

It is believed that proper oral hygiene and diet are the first steps toward the prevention of WSL. Other measures to control plaque accumulation could be recommended to those patients who exhibit high caries prevalence. ACP containing adhesive shows a promising effect on reducing the incidence of WSL irrespective of oral hygiene. Follow up clinical studies with longer duration are recommended as no previous study evaluated prolonged effect of using ACP containing adhesive in orthodontic treatment.

CLINICAL SIGNIFICANCE

Preserving morphology of the tooth is always a prime importance in dentistry. Demineralization of enamel can lead to many problems in the tooth and, hence, can be a limiting factor in the prognosis of the orthodontic treatment; ACP can be a promising agent in preventing enamel demineralization.

REFERENCES

1. Mitchell L. Decalcification during orthodontic treatment with fixed appliances – an overview. Br J Orthod 1992;19:199–205. DOI: 10.1179/bjo.19.3.199.
2. Derks A, Katsaros C, et al. Caries-inhibiting effect of preventive measures during orthodontic treatment with fixed appliances. A systematic review. Caries Res 2004;38:413–420. DOI: 10.1159/000079621.
3. Fornell AC, Skold-Larsson K, et al. Effect of a hydrophobic tooth coating on gingival health, mutans streptococci, and enamel demineralization in adolescents with fixed orthodontic appliances. Acta Odontol Scand 2002;60:37–41. DOI: 10.1080/000163502753471989.
4. O'Reilly MM, Featherstone JD. Demineralization and remineralization around orthodontic appliances: an in vivo study. Am J Orthod Dentofacial Orthop 1987;92:33–40. DOI: 10.1016/0889-5406(87)90293-9.
5. Gorelick L, Geiger AM, et al. Incidence of white spot formation after bonding and banding. Am J Orthod 1982;81:93–98. DOI: 10.1016/0002-9416(82)90032-x.
6. Lovrov S, Hertrich K, et al. Enamel demineralization during fixed orthodontic treatment-incidence and correlation to various oral-hygiene parameters. J Orofac Orthop 2007;68:353–363. DOI: 10.1007/s00056-007-0714-1.
7. Uysal T, Amasyali M, et al. Efficiency of amorphous calcium phosphate-containing orthodontic composite and resin modified glass ionomer on demineralization evaluated by a new laser fluorescence device. Eur J Dent 2009;3:127–134.
8. Mizrahi E. Enamel demineralization following orthodontic treatment. Am J Orthod 1982;82:62–67. DOI: 10.1016/0002-9416(82)90548-6.
9. Ogaard B, Rolla G, et al. Orthodontic appliances and enamel demineralization. Part 1. Lesion development. Am J Orthod Dentofacial Orthop 1988a;94:68–73. DOI: 10.1016/0889-5406(88)90453-2.
10. Boskey AL. Amorphous calcium phosphate: the contention of bone. J Dent Res 1997;76:1433–1436. DOI: 10.1177/00220345970760080501.
11. Cross KJ, Huq NL, et al. NMR studies of a novel calcium, phosphate and fluoride delivery vehicle-alpha(S1)-casein(59-79)by stabilized amorphous calcium fluoride phosphate nano complexes. Biomaterials 2004;25:5061–5069. DOI: 10.1016/j.biomaterials.2004.01.045.
12. Langhorst SE, O'donnell JN, et al. In vitro remineralization of enamel by polymermorphous calcium phosphate composite: quantitative micro radiographic study. Dent Mater 2009;25:884–891. DOI: 10.1016/j.dental.2009.01.094.
13. Uysal T, Amasyali M, et al. Amorphous calcium phosphate containing orthodontic composites. Do they prevent demineralization around orthodontic brackets? Aust Orthod J 2010a;26:10–15.
14. Chow CK, Wu CD, et al. In vitro properties of orthodontic adhesives with fluoride amorphous calciumphosphate. Int J Dent 2011;583521.
15. Zachrisson BU, Brobakken BO. Clinical comparison of direct versus indirect bonding with different bracket types and adhesives. Am J Orthod 1978;74:62–78.
16. Shen P, Cai F, et al. Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. J Dent Res 2001;80:2066–2070. DOI: 10.1177/00220345010800120801.
17. Reynolds EC, Cai F, et al. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. J Dent Res 2003;82:206–211. DOI: 10.1177/154405910308200311.
18. Uysal T, Amasyali M, et al. In vivo effects of amorphous calcium phosphate-containing orthodontic composite on enamel demineralization around orthodontic brackets. Aust Dent J 2010b;55:285–291. DOI: 10.1111/j.1834-7819.2010.01236.x.
19. Behnan SM, Arruda AO, et al. In vitro evaluation of various treatments to prevent demineralization next to orthodontic brackets. Am J Orthod Dentofacial Orthop 2010;138:712e1–712e7. DOI: 10.1016/j.ajodo.2010.05.014.
20. Richards N, Flaim GM, et al. Flexural strength and ion release of remineralizing resin restorative materials. J Dent Res 2007;86:2027.
21. Sukontapatipark W, El Agroudi MA, et al. Bacterial colonization associated with fixed orthodontic appliances. A scanning electron microscopy study. Eur J Orthod 2001;23:475–484. DOI: 10.1093/ejo/23.5.475.
22. Mizrahi E. Surface distribution of enamel opacities following orthodontic treatment. Am J Orthod 1983;84:323–331. DOI: 10.1016/s0002-9416(83)90348-2.
23. Artun J, Brobakken BO. Prevalence of carious white spots after orthodontic treatment with multibonded appliances. Eur J Orthod 1986;8:229–234. DOI: 10.1093/ejo/8.4.229