Original Research Article

A Comparative evaluation of the Shear bond strength of brackets bonded to pre-treated demineralised enamel treated with two different remineralising agents – An In-vitro study

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A B S T R A C T

Background: Remineralising agents are suggested on sound enamel and on demineralised enamel during fixed appliance therapy as they increase the risk of enamel demineralization; but their influence on bond strength is questionable. Toothmin has been recently introduced and showed promising results in preventing demineralization and promoting remineralization similar to CPP-ACP.Therefore, the rationale of doing this study is to evaluate Toothmin efficacy on shear bond strength (SBS) of pre-treated demineralized enamel.
Aims: To measure the SBS of brackets bonded to pretreated demineralised enamel treated with Toothmin and CPP-ACP.
Settings and Design: Teeth were divided into four groups prior to bonding. Group I with No enamel pre-treatment, Group II, demineralized enamel, Group III, CPP-ACP was applied on demineralized enamel whereas in Group IV, Tooth min was applied on demineralised enamel.
Methods and Materials: Intact enamel group, demineralised group and remineralised groups were evaluated for SBS and (Adhesive Remnant Index) ARI scores.
Statistical analysis: Data were analyzed using IBM SPSS version 20 software and subjedted to one way analysis of variance with Tukey's post hoc tests.
Results and Conclusions: Toothmin group had highest mean SBS (10.37±3.08) followed by CPP-ACP (9.6±2.43), control (8.67±2.31), and the demineralization (2.42±1.21) groups. Highest mean ARI scores were observed in Toothmin group (2.33±0.48) and the least in the demineralization group (0.4±0.507).Demineralization of enamel significantly reduces the SBS of orthodontic brackets. Remineralization of enamel with Toothmin or CPP-ACP after demineralisation improved bond strength. Highest mean ARI scores were observed in Toothmin group and lowest in demineralized group.

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1. Introduction

Fixed orthodontic appliances create problems in self-cleansing resulting in increased plaque retention and biofilm accumulation.[1–5] Studies shows that fixed orthodontic appliances compromises patients compliance towards oral hygiene maintenance.[6] and increases the risk of enamel demineralization.[7–10]

Gorelick et al.[9] and Boersma et al.[11] reported the occurrence of early enamel lesions in 11- 24% of patients before orthodontic therapy and non developmental early enamel lesions in 50% of patients undergoing fixed orthodontic treatment. Tufekci et al[12] reported steady progress in the formation of WSLs from early months of orthodontic treatment to later stages.

Topical application of CPP-ACP paste and fluoride varnish, laser with fluoride,[13] laser with CPP-ACP,[14]

laser with CPP-ACPF,[15] low-viscosity light-curing resin infiltration into deeper layers are the factors that can effect enamel lesions during fixed appliance therapy.[16]

Due to the increased occurence of enamel lesions during fixed appliance therapy, remineralising agents are suggested on sound enamel and on demineralised enamel but their influence on bond strength is questionable.

Several in vitro studies evaluated SBS after treatment of enamel surface with different preventive measures.[17] Previous literature revealed variable results like significant increase[18,19] or decrease[20] or no variation in the SBS after pretreatment.[21,22]

Toothmin has been recently introduced and showed promising results in its efficiency in preventing demineralization and promoting remineralization during orthodontic therapy similar to CPP-ACP.[23] CPP-ACP reduces demineralisation and promotes remineralisation by maintaining higher concentrations of calcium and phosphate mineral ion content on tooth surface. Toothmin toothpaste arrests the caries activity by maintaining higher concentrations of soluble calcium and phosphate ions which aids in better adsorption to the enamel surface.

Although it was proven that Toothmin is as effective as CPP-ACP in remineralising surface enamel.[23] however clinical data suggesting its effect on SBS of brackets attached to conditioned demineralised enamel surface is lacking. Therefore, the rationale of doing this in – vitro study is to evaluate the results of Toothmin and CPP-ACP paste application on SBS of pre-treated demineralized enamel.

From the previous research, the effect size was calculated to be 0.57 which is used to find out the sample size using G Power software. Sample size estimated to be 56 (rounded to 60) at a confidence interval of 95% and power of the study 95%. Based on 1:1 ratio 15 teeth per group was selected.

Teeth with good crown structure were included and teeth with decalcified enamel and which have undergone pre-treatment with surface reagents were excluded from the study.

Extracted premolars were stored in 0.1% thymol solution. Soft tissue remnants and callus was removed with a scaler. Each tooth was affixed vertically in a self-cure acrylic block so that the crown is exposed. Teeth were divided into four groups of 15 teeth each, as described in the following sections (Figure 1).

Group I	No enamel pre-treatment (Control)
Group II	Demineralisation of enamel
Group III	CPP-ACP paste applied to demineralised enamel
Group IV	Toothmin applied to demineralised enamel

To establish a baseline, equal amount of mineral content of individual tooth was confirmed by a portable battery-powered laser fluorescence device DIAGNOdent Pen.

Diagnodent readings were recorded for the groups I, II, III and IV to determine mineral content.

2. Demineralisation Process

The demineralization procedure was followed according to Wei Hua and John D. B. Featherstone[10]. With this procedure sequential demineralization and remineralization were performed in order to mimic the remineralizing stage of the caries process. Each premolar was kept in demineralization solution with acidic pH of 4.3 for 6 hours at 37^0C later washed with deionized water, and again kept in remineralization solution with neutral pH of 7.0 for about 18 hours. This was repeated daily for 3 weeks and on 21st day, teeth were dried and evaluated for frosty white appearance of enamel.

After demineralization, teeth were segregated into group III and IV. Mineral content was confirmed with the DIAGNOdent Pen and then stored in artificial saliva for around 30 days.

1. **Group I:** No enamel pre-treatment was done in this group.
2. **Group II:** In this group, bonding done on demineralized enamel.
3. **Group III:** In this group, application of CPP-ACP paste on demineralized enamel (Figure 2) was done prior to bonding. A cyclic application of CPP-ACP paste for 5 minutes followed by deionized water rinse for every 6 hours. Same procedure repeated for remineralisation process. A total of 10 cycles was done. During these cycles, teeth were stored in artificial saliva. Once demineralisation and remineralisation were done alternatively, brackets were bonded on the tooth surface.
4. **Group IV:** In this group, same protocol was followed as above instead, Tooth min (Figure 2) was used for CPP-ACP.
5. After remineralization of groups III and IV, Diagnodent readings (Figure 3) were taken to match the scores in both the groups.

2.1. Bracket bonding

Sixty stainless steel pre-adjusted edgewise maxillary bicuspid brackets with 0.022-inch slots (3M UNITEK) were adopted in this study. The buccal surfaces were cleansed for 5 seconds, washed for 10 seconds, and finally dried for about 10 seconds using an air-water syringe. Tooth surface etched with 37% Ortho-phosphoric acid followed by water rinse and then oil-free air applied for 10 sec till the surface appears frosty white. After conditioning with Primer, stainless-steel brackets were bonded to premolars with Trans bond XT and cured with LED. The specimens were kept in deionized water in a sealed container at room temperature for one day before debonding.

2.2. Debonding procedure and Shear bond strength testing

Brackets were subjected to debonding on Instron Testing Machine (Figure 4) and SBS was calculated for all the specimens. SBS obtained in Newtons from Insytron machine was converted to MPA by dividing the Force in Newton by Area of the bracket base (mm2).

3. Adhesive Remnant Index

A digital microscope with ×50 magnification was used to determine the mode of failure and the extent of remaining composite on the enamel (Figure 5). ARI scores at the failure area were recorded similar to the study by Artun and Bergland.[24]

 The Adhesive Remnant Index
 Index score Enamel Adhesive Remnant

1. No adhesive left on the enamel
2. Less than 50% of adhesive left on the enamel
3. More than 50% of adhesive left on the enamel
4. All adhesives left on the enamel

3.1. Statistical analysis

Data were analyzed using IBM SPSS version 20 software (IBM SPSS, IBM Corp., Armonk, NY, USA). Descriptive statistics, one way analysis of variance with Tukey's post hoc tests, chi square test, Mann Whitney U tests, and Friedman's tests were done to analyze the study data.

4. Results

resents the descriptive statistics for shear bond strength in the study groups. It was found that Toothmin group had highest mean shear bond strength (10.37±3.08) followed by CPP-ACP (9.6±2.43), control (8.67±2.31), and the demineralization (2.42±1.21) groups. These differences between groups were statistically significant (Table 2). In multiple pairwise comparisons, it was observed that the demineralization group had significantly lower shear bond strength compared to all other groups. While there was no statistically significant difference between CPP-ACP and Toothmin groups, both the groups demonstrated significantly higher shear bond strength compared to the control group (Table 3).

 hows the comparison of ARI scores between the study groups. While ARI score 0 was most common in the demineralization group, score 2 was most common in the remaining three groups, the difference of which was statistically significant. Table 5 presents the descriptive statistics for mean ARI scores in the study groups. Highest mean ARI scores were observed in Toothmin group (2.33±0.48) and the least mean score in the demineralization group (0.4±0.507). These differences between mean ARI scores were statistically significant (Table 6). In post hoc

analysis, demineralization group demonstrated significantly lesser mean ARI scores compared to all the other three groups. No other pairwise comparisons were statistically significant.

Figure 1: Teeth divided into four groups

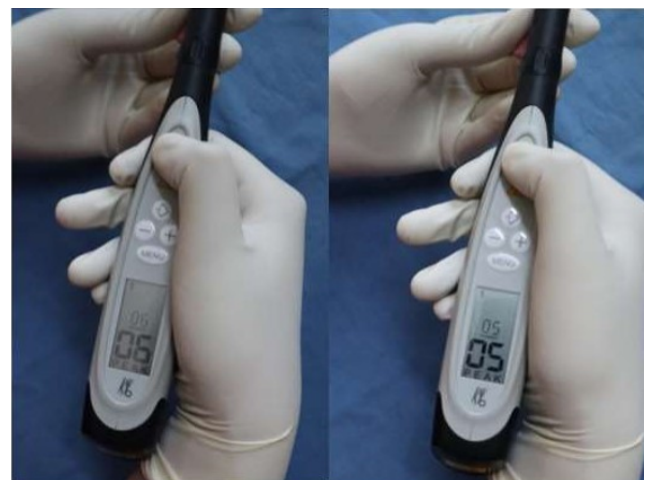**Figure 2:** Application of Remineralisation pastes

Figure 3: Diagnodent scores of Remineralised teeth

5. Discussion

Development of White Spot Lesions (WSLs) is most commonly seen before and during fixed orthodontic

Table 1: Descriptive statistics for shear bond strength in the study groups

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Control	15	8.6760	2.31508	.59775	7.7940	10.3580	6.41	13.95
Demineralization	15	2.4293	1.21092	.31266	1.7587	3.0999	1.47	4.83
CPP-ACP	15	9.6080	2.43913	.62978	7.7573	10.4587	6.13	13.95
Toothmin	15	10.3793	3.08628	.79688	8.6702	12.0885	7.18	17.68

Table 2: Comparison of shear bond strength between the study groups

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		F value	P value
					Lower Bound	Upper Bound		
Control	15	8.6760	2.31508	.59775	7.7940	10.3580		
Demineralization	15	2.4293	1.21092	.31266	1.7587	3.0999	34.82	<0.001*
CPP-ACP	15	9.6080	2.43913	.62978	7.7573	10.4587		
Toothmin	15	10.3793	3.08628	.79688	8.6702	12.0885		

One way analysis of variance; $p \leq .05$ considered statistically significant; * denotes significance

Table 3: Multiple pairwise comparisons of shear bond strength between the study groups

Reference Group	Comparison Group	Mean Difference	P value	95% Confidence Interval	
				Lower Bound	Upper Bound
	Demineralization	6.24667*	.000	4.3637	8.9296
Control	CPP-ACP	-.93200	.041*	-2.3149	-0.216
	Toothmin	-1.70333	.029*	-3.5863	-0.9796
	CPP-ACP	-7.17867*	.000	-8.9616	-4.3957
Demineralization	Toothmin	-7.95000*	.000	-10.2329	-5.6671
CPP-ACP	Toothmin	-0.67133	.459	-3.5543	1.0116

Tukey's post hoc tests; $p \leq .05$ considered statistically significant; * denotes significance

Table 4: Comparison of ARI scores between the study groups

Group	ARI Score				P value
	0	1	2	3	
Control	0	2 (13.3)	7 (46.7)	6 (40)	
Demineralization	9 (60)	6 (40)	0	0	<0.001*
CPP-ACP	0	0	11 (73.3)	4 (26.7)	
Toothmin	0	0	10 (66.7)	5 (33.3)	

Chi square test; $p \leq .05$ considered statistically significant; * denotes significance

Table 5: Descriptive statistics for ARI scores in the study groups

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Control	15	2.27	.704	.182	1.88	2.66	1	3
Demineralization	15	.40	.507	.131	.12	.68	0	1
CPP-ACP	15	2.27	.458	.118	2.01	2.52	2	3
Toothmin	15	2.33	.488	.126	2.06	2.60	2	3

Table 6: Comparison of mean ARI scores between the study groups

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		F value	P value
					Lower Bound	Upper Bound		
Control	15	2.27	.704	.182	1.88	2.66		
Demineralization	15	.40	.507	.131	.12	.68	44.64	<0.001*
CPP-ACP	15	2.27	.458	.118	2.01	2.52		
Toothmin	15	2.33	.488	.126	2.06	2.60		

One way analysis of variance; p≤.05 considered statistically significant; * denotes significance

Figure 4: Shear bond strength testing on Instron machine
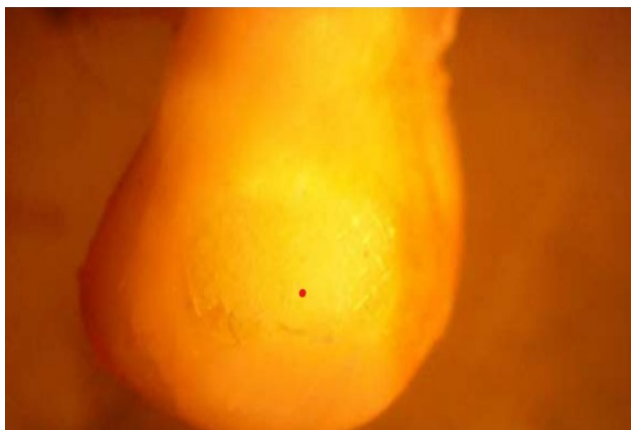
Figure 5: Adhesive Remnant Index score

treatment and their prevention is extremely important. Several authors reported prevalence of WSLs before orthodontic therapy to the extent of 11 to 24%.[9,11] Use of pre-treatment agents in remineralizing these lesions would be beneficial because of high prevalence of WSLs (30-70%) during fixed orthodontic treatment;[25–27] but the effect of these agents on SBS of brackets is debatable.

Ideally, the bonded brackets should have bond strength ample enough to withstand the orthodontic and masticatory forces throughout the treatment duration but should be debonded at the end of the treatment with no damage to the tooth.[28]

In this study SBS of orthodontic brackets were tested on Intact enamel, after demineralization and after applying two different remineralising agents, CPP-ACP and Tooth Min. It was observed that Toothmin group had highest mean shear bond strength followed by CPP-ACP, Control, and the demineralization groups. There was no statistically significant difference between CPP-ACP and Toothmin groups, but demonstrated significantly higher shear bond strength in comparison with the control group.

To establish a baseline, equal amount of mineral content of each tooth was confirmed by laser fluorescence device DIAGNOdent Pen. Diagnodent readings were noted for the group I (Intact enamel), group II (Demineralised enamel), group III (CPP-ACP) and group IV (Toothmin) to determine mineral content. There was significant increase in the diagnodent scores following demineralization which reduced significantly after the application of remineralizing agent in both the CPP-ACP and the Toothmin groups which indicates the identical remineralisation capacity of both CPP-ACP and Toothmin.

Several methods for artificial demineralization of teeth are available to imitate primary caries lesions. For this purpose, Farhadian et al.[29] used the microbiological method, Baka et al.[30] and Veli et al.[31] used immersion in a demineralizing solution, and Uysal et al.[32] and Ekizer et al.[33] utilized a pH cycle. Marquezan et al.[34]

compared three tooth demineralization methods, which includes the use of an acid gel, a pH cycle, and microbiological method and concluded that the use of a pH cycle was more effective than an acid gel. Hence in the current study, pH cycle advocated by Hu and Featherstone[10]

was adopted. 21 days of pH cycling[32] resulted in good frosty enamel surface in contrast to 14 days cycle.[10] Artificial caries lesions are quite similar but not identical to natural caries lesions which aids in the possibilty of testing invitro.[35]

Extracted teeth are routinely stored in aqueous solutions till the required number were collected in Invitro studies.[36] Habelitz et al[37] study observed, a 25% reduction in the hardness of tooth when they were stored in distilled water for one day. According to Aydin et al.[36] study, 0.1% Thymol solution as storage medium is best in maintaining the mineral content of the enamel. Therefore, they were stored in Thymol solution till all the teeth were collected in the current study. In between the cycles of demineralisation and remineralisation, teeth were kept in artificial saliva to simulate the oral environment.

Current study observed least SBS in demineralized specimens compared to other groups which can be accredited to the poor quality of the surface enamel and the lack of resin tags that form the mechanical interlock. Application of adhesive resin after enamel demineralization led to evenly distributed rows of tags.[38] Highest SBS was observed in Toothmin group followed by CPP-ACP followed by Intact Enamel group and demineralised group the least SBS with statistically significant difference. Highest mean bond strength observed in Toothmin group compared to CPP-ACP could be the result of the availability of greater mineralized ions in a soluble form at high concentrations in Toothmin providing the increased mineralized content thereby increased SBS. Incipient enamel lesions occur by loss of minerals beneath the surface layer.[39] Since enamel minerals are effective in establishing a good bond,[40] this factor decreases SBS of brackets to demineralized enamel. Current study results were acceptable as SBS values of all the groups were higher than the Reynold[41] suggested values (5.9– 7.8 MPa) except in demineralisation group.

In Keles and P. Daneshkazemi[42] studies higher SBS of brackets were found in intact enamel groups than demineralized enamel groups with significant difference between them.In Uysal[32] study, SBSs of CPP-ACP treated samples were higher compared to the demineralization group, and there were no significant differences between the control and CPP-ACP treated groups. It would be reasonable to think that CPP-ACP led to an identical enamel surface composition as observed in the control group.

Similar to the present study findings, Kecik et al.[43] and Xianojun et al.[44] found that the SBS of CPP-ACP treated samples were higher than that of the control group.Quite contrast to the other studies, in Gulec and Goymen[45] study no statistically significant difference was observed in SBS of intact enamel compared to demineralized enamel. These differences in SBS in various studies could be due to usage of different bonding materials, prolonged use of remineralising agents and storage time of teeth in artificial saliva.

To overcome the short comings of other studies[45] differences in tooth demineralisation protocols and no standardization of demineralization quantification,DIAGNOdent device was used in the present study to measure the quantity of mineral content in all the groups.

The amount of remaining adhesive on the enamel surface after bracket debonding is routinely evaluated by Adhesive remnant index.[46] But the efficiency of the ARI to reflect the bond strength is questionable.[31,47–51] The lesser the adhesive remnant on the enamel post debonding, the safer the enamel cleanup.[49,52] However lesser the risk of enamel fracture during bracket removal with the presence of minimal composite remaining at the enamel surface.[50–53]

In this study, highest mean ARI scores were observed in Toothmin group and the least mean score in the demineralization group with statistically significant difference. In contrast to the present study, Nabawy et al.[54] study showed no statistically significant differences regarding ARI scores between the 3 groups where most of the scores were either 1 or 2 in all the groups, which indicates a cohesive failure.[55] Cohesive failures offer the enamel surface protection by reducing the possible risk of enamel fracture during debonding as well as enamel damage during enamel clean-up post debonding.

Similar to the current study, Cossellu et al.[49] and Uysal et al[32] studies also reported that groups with greater bond strength often exhibit ARI score of 1 and 0 respectively. However Ekizerstudy[33] reported recurrent ARI scores of 2 and 3 in demineralized group and APF gel treated demineralized enamel group respectively.

Variations in demineralization procedures, rate of thermal cycling, the rod movement speed in Instron machine,storage environment, pre-treatment agents application methods and the bracket base used[42] would have affected results from different studies. In order to replicate the oral environment, extracted human premolars teeth were used in the current study rather than bovine teeth which were shown to have two or three times faster dissolving capacity than human enamel,[56] and teeth were stored in artificial saliva.[57,58]

However, it is not possible to replicate the oral environment in in-vitro studies which is considered as the major limitation of the current study. Therefore it is preferable to conduct in-vivo studies to test the failure rate of orthodontic brackets bonded after CPP–ACP application and Toothmin.

6. Results concluded from the present study were as follows:

1. Demineralization of enamel significantly reduces the SBS of orthodontic brackets.

2. Remineralization of enamel with Toothmin or CPP-ACP after demineralisation improved bond strength.

3. Both Toothmin and CPP-ACP pretreatment are equally efficient as remineralising agents and for bonding orthodontic brackets.

4. Highest mean ARI scores were observed in Toothmin group with lesser failures at the composite-enamel interface and lower ARI scores with highest failures at composite-enamel interface were observed in untreated demineralized group.

7. Source of Funding

None.

8. Conflict of Interest

None.

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