

## Effects of demineralization-inhibition procedures on the bond strength of brackets bonded to demineralized enamel surface

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**Objective:** To study and compare the effects of different demineralization-inhibition methods on the shear bond strength (SBS) and fracture mode of an adhesive used to bond orthodontic brackets to demineralized enamel surfaces. **Methods:** Eighty freshly extracted, human maxillary premolars were divided into 4 equal groups and demineralized over the course of 21 days. Brackets were bonded to the demineralized enamel of teeth in Group 1. In Group 2, bonding was performed following resin infiltration (ICON<sup>®</sup>, DMG, Hamburg, Germany). Before bonding, pre-treatment with acidulated phosphate fluoride (APF) or solutions containing casein phosphopeptide-amorphous calcium phosphate with 2% neutral sodium fluoride (CPP-ACP/wF) was performed in Groups 3 and 4, respectively. The SBS values of the brackets were measured and recorded following mechanical shearing of the bracket from the tooth surface. The adhesive remnant index (ARI) scores were determined after the brackets failed. Statistical comparisons were performed using one-way ANOVA, Tukey's post-tests, and G-tests. **Results:** Significant differences were found in some of the intergroup comparisons of the SBS values ( $F = 39.287, p < 0.001$ ). No significant differences were found between the values for the APF-gel and control groups, whereas significantly higher SBS values were recorded for the resin-infiltrated and CPP-ACP/wF-treated groups. The ARI scores were also significantly different among the 4 groups ( $p < 0.001$ ). **Conclusions:** Tooth surfaces exposed to resin infiltration and CPP-ACP/wF application showed higher debonding forces than the untreated, demineralized surfaces.

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## INTRODUCTION

Enamel decalcification or demineralization is a significant clinical problem encountered during orthodontic treatment.<sup>1</sup> Orthodontic treatment with fixed appliances complicates maintenance of oral hygiene and increases the risk of enamel lesion development.<sup>2-5</sup> In studies comparing orthodontically treated and untreated individuals, the incidence of enamel white-spot lesions was higher (incidences of 11.7%,<sup>2</sup> 16%,<sup>3</sup> and 25.6%<sup>4</sup>) in patients who received orthodontic treatment. Other reports have indicated that up to 50% of patients undergoing fixed orthodontic treatment may exhibit non-developmental white-spot lesions,<sup>4</sup> with teenagers being at a higher risk than adults.<sup>5</sup> However, white-spot lesions may also develop in individuals who have not received orthodontic treatment. For example, Boersma et al.<sup>6</sup> reported white-spot lesions in 11% of orthodontically untreated subjects, and Gorelick et al.<sup>4</sup> reported that 24% of the patients had white-spot lesions before orthodontic therapy. These findings raise concerns over the possibility that orthodontic treatment may be performed in individuals with existing white-spot lesions.

Administration of topical agents containing fluoride or casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), maintenance of oral hygiene, and dietary control have been suggested as mechanisms to control the formation of enamel lesions during fixed-appliance treatment.<sup>7</sup> In contemporary orthodontic literature, fluoride and CPP-ACP applications are accepted approaches for remineralizing previously demineralized enamel. Fluoride ions in plaque immediately promote remineralization by formation of fluorapatite.<sup>8</sup> In addition, fluoride application can promote remineralization of previously demineralized enamel in cases where adequate amounts of calcium and phosphate ions are available.<sup>9</sup> The anticariogenic activity of CPP-ACP has also been demonstrated in laboratory, animal, and human experiments.<sup>9-11</sup> Additionally, this treatment has been demonstrated to significantly increase the levels of calcium and phosphate ions in supragingival plaque, thereby promoting the remineralization of enamel subsurface lesions in situ.<sup>12</sup> Nevertheless, in noncompliant individuals, these strategies have considerable limitations.<sup>13</sup> For example, remineralization therapy fails when plaque is not sufficiently removed (due to either lack of cooperation or accessibility).<sup>14</sup>

The current approach in preventive dentistry, prevention of initial enamel carious lesions may be achieved through the use of enamel-penetrating, light-cured resins.<sup>15,16</sup> The porosity of untreated enamel lesions is believed to facilitate the diffusion of acids and dissolved minerals. Therefore, the aim of enamel infiltration is to occlude these pores, and thus prevent acid penetration into the lesions by forming a diffusion barrier within the enamel

lesion. Infiltration of the enamel structure by the resin matrix may also strengthen the tooth enamel, preventing caries formation or progression for years.

In routine orthodontic practice, it is also important to achieve a reliable adhesive bond between the orthodontic appliance and the tooth enamel. Therefore, the purpose of this research was to compare the effects of 3 different demineralization-inhibition procedures (resin infiltration, application of acidulated phosphate-sodium fluoride [APF] or CPP-ACP with 2% neutral sodium fluoride [CPP-ACP/wF]) on the shear bond strength (SBS) of orthodontic brackets bonded to pretreated, demineralized enamel. The mode of the eventual bond failure was also determined using a modified adhesive remnant index (ARI).

## MATERIAL AND METHODS

A power analysis conducted using the G\*Power ver. 3.0.10. software (Franz Faul, Universität Kiel, Germany) indicated that, with a group ratio of 1:1, a total sample size of 76 teeth would give more than 80% power (actual power = 0.8234; number of groups = 4) to detect significant differences with an effect size of 0.40 at an  $\alpha = 0.05$  level of significance (critical F = 2.7318; noncentrality parameter  $\lambda = 12.1600$ ).

Eighty caries-free and intact maxillary premolars were extracted, for orthodontic reasons, from patients aged 12-16 years and stored in distilled water at room temperature until use (maximum 1 month). Teeth with hypoplastic areas, cracks, or gross irregularities in the enamel structure were excluded from the study. The criteria for tooth selection dictated no pre-treatment with a chemical agent, such as alcohol, formalin, or hydrogen peroxide. Soft tissue remnants and calculi were removed from the teeth and they were cleaned with a fluoride-free pumice and rubber cup.

### Demineralization procedure

The crown surface of each tooth was painted with an acid-resistant varnish (Enamel Pro<sup>®</sup> Varnish; Premier Dental, Plymouth Meeting, PA, USA), leaving an exposed window of enamel (approximately, 3 × 4 mm) on the middle third of the buccal surface. This resulted in most of the tooth crown being covered by an acid-resistant varnish, with only the exposed enamel available to be attacked by acid. The daily pH cycling procedure included a demineralization period of 6 hours and a remineralization period of 18 hours.<sup>17</sup> Each crown was individually immersed in 40 mL of a demineralization solution containing 2.0 mmol/L calcium, 2.0 mmol/L phosphate, and 75 mmol/L acetate at pH 4.3 for 6 hours at 37°C. At the conclusion of the demineralization period, the specimens were rinsed with deionized water and

individually immersed in 20 ml of the remineralization solution at 37°C overnight (18 hours) to simulate the remineralization stage of the caries process.<sup>17</sup> The remineralization solution consisted of 1.5 mmol/L calcium, 0.9 mmol/L phosphate, 150 mmol/L potassium chloride, and 20 mmol/L cacodylate buffer at pH 7.0. This cycling procedure was repeated daily for 21 days.

#### **Demineralization-inhibition treatment**

The teeth were distributed into 4 equal groups of 20 teeth: 3 experimental and 1 control group. No enamel treatment was performed on Group 1 (the control group) teeth after completion of the demineralization procedure. The remaining 3 groups were identified according to the method employed for inhibiting demineralization. Enamel lesions in Group 2 teeth were treated with a resin infiltrant (ICON<sup>®</sup>; DMG, Hamburg, Germany) according to the manufacturer's recommendations, before bonding the brackets to the tooth surfaces. Demineralized teeth in Group 3 were treated in a saturated solution of 1.23% APF (DFL, Petropolis, Brazil) for 4 days.<sup>18</sup> The saturated solution of APF (1 - 7%) was prepared in distilled water. The teeth in Group 4 were exposed to CPP-ACP/wF (Enamel Pro<sup>®</sup> Gel; Premier Dental, Plymouth Meeting, PA, USA) for 4 days.<sup>18</sup> The saturated CPP-ACP/wF solution (1 - 4%) was also prepared in distilled water.

#### **Bonding procedure**

Before bonding the orthodontic brackets to the enamel surfaces, the teeth in Groups 3 and 4 were rinsed with water for 15 seconds and dried with oil-free air for 10 seconds to remove remnants of the APF and CPP-ACP/wF solutions from the buccal surfaces. A 37% orthophosphoric acid gel (3M ESPE, St. Paul, MN, USA) was used to acid etch all the teeth for 15 seconds. The teeth were rinsed with water for 15 seconds and dried with oil-free air for 10 seconds until the etched enamel showed a frosty white appearance. After preparation of the enamel surfaces, the liquid primer Transbond XT (3M Unitek, Monrovia, CA, USA) was applied to the etched surface, and not cured, according to the manufacturer's recommendation. Transbond XT adhesive was also applied to each bracket base, and the bracket was placed onto the tooth in the center of the crown, with the center of the bracket over the long axis of the tooth. Excess resin was removed with an explorer before polymerization. A quartz-tungsten halogen light unit (Hilux 350, Express Dental Products, Toronto, Canada) with a 10-mm diameter light tip was used for 40 seconds to cure the adhesive (20 seconds from the mesial and 20 seconds from the distal sides). The specimens were then stored in distilled water, at 37°C, for 24 hours before SBS testing.

#### **Debonding procedure**

Each tooth was secured in a jig attached to the base plate of an Instron Universal Testing Machine (Model 1135-5; Instron Corp., Norwood, MA, USA). A chisel-edge plunger was mounted in the movable crosshead of the testing machine and positioned to apply a shear force to the enamel-resin interface. A crosshead speed of 0.5 mm/minute was used, and the maximum load necessary to debond the bracket was recorded. The force required to debond each bracket was measured in Newton (N), and the SBS (1 megapascal [MPa] = 1 N/mm<sup>2</sup>) was then calculated by dividing the force values by the bracket base area (12 mm<sup>2</sup>).

#### **Residual adhesive**

After debonding, all teeth and brackets were microscopically (Model 5240; Olympus, Tokyo, Japan) evaluated at ×10 magnification by 1 operator (A.E.), who was blinded to the group allocations, to determine ARI scores.<sup>19</sup> The ARI scores were used as a comprehensive means of defining the sites of bond failure between the enamel, resin, and bracket base. The test yielded 4 possible scores: 0, no adhesive remained on the tooth; 1, less than half of the adhesive remained on the tooth; 2, more than half of the adhesive remained on the tooth; 3, all of the adhesive remained on the tooth, with a distinct impression of the bracket mesh.

#### **Statistical methods**

All statistical analyses were performed using the Statistical Package for SPSS for Windows 13.0 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov normality test and Levene's variance homogeneity test were applied to the data. The data were found to be normally distributed, and there was homogeneity of variance among the groups. Thus, the statistical evaluation was performed using parametric tests (one-way ANOVA, followed by Tukey's post-hoc test). The means, standard deviations, and minimum and maximum values were also calculated for each group. The G-test was used to determine significant differences among the ARI scores from the 4 groups. A *p*-value of less than 0.05 was considered statistically significant in all tests.

## **RESULTS**

According to Kruskal-Wallis analysis of variance, statistically significant differences were present in some of the intergroup comparisons ( $F = 39.287$ ;  $p < 0.001$ ). The highest SBS values were found in the resin infiltration (mean:  $20.6 \pm 4.4$  MPa) and CPP-ACP/wF application (mean:  $19.8 \pm 0.7$  MPa) groups; these values were found to be significantly greater than the SBS values in the control (mean:  $12.3 \pm 1.3$  MPa) and APF gel (mean:  $9.9 \pm 2.2$

**Table 1.** Descriptive statistics and the results of ANOVA and Tukey's tests comparing shear bond strengths in the 4 groups tested

Groups	N	Remineralization procedure	Shear bond strength				ANOVA	Multiple comparison (Tukey test)		
			Mean (MPa)	SD	Min	Max	F = 39.287	Sig.	Group 2	Group 3
Group 1	20	No (control)	12.3	1.3	5.3	18.8		$p < 0.001$	NS	$p < 0.001$
Group 2	20	Resin infiltration	20.6	4.4	13.1	27.7	$p < 0.001$		$p < 0.001$	NS
Group 3	20	APF-Gel	9.9	2.2	6.9	15.0				$p < 0.001$
Group 4	20	CPP-ACP/wF	19.8	0.7	10.1	29.9				

N, Sample size; SD, standard deviation; Min, minimum; Max, maximum; Sig., significance; APF, acidulated phosphate-sodium fluoride; CPP-ACP/wF, casein phosphopeptide-amorphous calcium phosphate with 2% neutral sodium fluoride; NS, not significant.

**Table 2.** Frequency distribution of the adhesive remnant index (ARI) scores of the groups<sup>†</sup>

Groups*	N	Remineralization procedure	ARI scores, n (%)				Significance (G test)
			0	1	2	3	
Group 1 <sup>a</sup>	20	No (control)	1 (5)	5 (25)	6 (30)	8 (40)	
Group 2 <sup>b</sup>	20	Resin infiltration	2 (10)	5 (25)	10 (50)	3 (15)	$p < 0.001$
Group 3 <sup>c</sup>	20	APF-Gel	0 (0)	3 (15)	8 (40)	9 (45)	
Group 4 <sup>d</sup>	20	CPP-ACP/wF	7 (35)	12 (60)	1 (5)	0 (0)	

N, Sample size; n, number; APF, acidulated phosphate-sodium fluoride; CPP-ACP/wF, casein phosphopeptide-amorphous calcium phosphate with 2% neutral sodium fluoride. \*Different letters show statistically significant differences. <sup>†</sup>ARI scores: 0, no adhesive remaining on the enamel surface; 1, less than 50% adhesive remaining on tooth; 2, more than 50% adhesive remaining on tooth; 3, all adhesive remaining on tooth surface.

MPa) groups (Table 1). The SBS scores between Groups 1 and 3 ( $p = 0.646$ ), and between Groups 2 and 4 ( $p = 0.995$ ) were not significantly different from each other.

The results of the G-test comparisons indicated that there were significant differences among the 4 groups tested (Table 2,  $p < 0.001$ ). There was a greater frequency of ARI scores of 2 and 3 in Groups 1 and 3. In Group 2, the failures were mostly cohesive, and some adhesive failures within the resin were also observed. In Group 4, there was a higher frequency of ARI scores of 0 and 1.

## DISCUSSION

In this study, lesions were created in the enamel surfaces of teeth, which were then treated with various methods to inhibit further demineralization. After treatment, stainless steel orthodontic brackets were bonded to the lesions and subjected to mechanical shearing; the SBS and type of bond fracture that resulted in the removal of the bracket was determined. The results suggest that APF gel does not significantly affect the bond strength of orthodontic brackets bonded to demineralized enamel,

relative to that for an untreated tooth surface. The results of the present study agree with previous findings<sup>20-22</sup> that suggested that the topical administration of fluoride does not significantly alter enamel bond strength. The presence of fluoride on the tooth surface has been considered to potentially lower the surface energy of the adherent, decreasing the ability of the adhesive to spread. However, the bond strength appears to be unaffected whether or not a fluoridated solution was used.<sup>23</sup> The present study also demonstrated that there was no affect of APF-containing gel on SBS when the brackets were bonded to a demineralized enamel surface. In contrast, CPP-ACP/wF gel and resin-infiltrated enamel significantly increased the SBS of the orthodontic brackets.

The development of ACP materials and their incorporation into dentistry is the result of an approach that aims to reverse the effects of demineralization on enamel surfaces.<sup>24</sup> In the literature on orthodontic treatment, Dunn<sup>24</sup> and Foster et al.<sup>25</sup> compared the SBS of orthodontic brackets following their application to tooth surfaces using ACP-containing adhesive and compared the results to those obtained with brackets bonded to

tooth surfaces using conventional orthodontic adhesives. They reported that the ACP adhesive had a low, but satisfactory, bond strength that allowed it to function as an orthodontic adhesive. Uysal et al.<sup>26</sup> also evaluated the SBS of an ACP-containing orthodontic adhesive relative to conventional composite materials used as orthodontic lingual retainer adhesives and found that ACP-containing Aegis<sup>®</sup> Ortho (Bosworth Co., Skokie, IL, USA) adhesive, resulted in a significant decrease in the bond strength to the etched enamel surface.

Until recently, dentists and oral hygienists had only 2 principal options for treating white-spot lesions; fluoride or remineralization therapies. Resin infiltration is a major breakthrough in micro-invasive technology that will fill, reinforce, and stabilize demineralized enamel, as suggested by previous *in situ* results indicating a reduced progression of infiltrated lesions when compared to the results in untreated controls.<sup>27</sup> In Group 2 of this study, resin infiltration was performed using pre-product materials and applicators. More recently, a kit for resin infiltration (ICON, DMG) became commercially available and includes materials similar to those used in the present study, where the highest SBS results were observed. The results may be explained by the fact that demineralized enamel allows penetration of the resin, resulting in increased micromechanical interdigitation, which is the most important part of enamel bonding. These positive effects on SBS are also likely due to the structure of the orthodontic bonding composite. Therefore, it is questionable whether superficial smooth-surface sealing with these resins is, as yet, generally applicable to daily practice. Compared with the other techniques for treating demineralization, the infiltration treatment might possess several advantages. Resin infiltration of the porous lesion structures might strengthen the lesion mechanically, helping to prevent caries formation. Moreover, this method may be used with patients with a known sensitivity to fluoride.

Failure within the bracket-resin-enamel complex may occur within the bracket, between the bracket and the resin, within the resin, and between the tooth surface and the resin. Bond failure at the bracket-resin interface or within the resin is more desirable than at the resin-enamel interface. In the present study, there was a greater frequency of ARI scores of 2 and 3 in Groups 1 and 4, indicating that the failures were mainly at the resin-bracket interface. In Group 2, the failures were mostly cohesive, and some adhesive failures within the resin were also observed. In Group 3, there was a higher frequency of ARI scores of 0 and 1, indicating adhesive failures within the resin.

The findings of this study reinforce the need for CPP-ACP/wF gel therapy or resin infiltration therapy in patients who have white-spot lesions before orthodontic

bonding. In contrast to this result, APF gel application is not acceptable as it does not contribute to an increase in the SBS scores of orthodontic brackets attached to teeth with enamel lesions.

## CONCLUSION

Within the limitations of an *in vitro* setting, the following clinical conclusions can be drawn:

1. To reduce bonding failure during fixed orthodontic treatment, resin infiltration materials and CPP-ACP/wF gels may safely be used as prophylactic agents before brackets are bonded to patients with enamel lesions.
2. In comparison with the conventional method, application of the APF-containing gel did not increase the bond strength of brackets bonded to a demineralized enamel surface.
3. Further investigation is needed to evaluate the clinical performance and to better understand any possible adverse effects caused by the newly developed gels and resin infiltration materials before their introduction into routine clinical practice.

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