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ORIGINAL ARTICLE

Can demineralized enamel surfaces be bonded safely?

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Abstract

Objective. To evaluate and compare the effects of enamel demineralization, microabrasion therapy and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) application on the shear bond strength (SBS) of orthodontic brackets bonded to enamel surfaces and enamel color. ***Materials and methods.*** Eighty freshly extracted human maxillary premolar teeth were allocated to one of the four groups. Brackets were bonded directly to non-demineralized enamel surfaces in Group I (control group), directly to the demineralized enamel surfaces in Group II, to demineralized enamel surfaces after CPP-ACP application in Group III and to demineralized enamel surfaces after microabrasion therapy in Group IV. The samples were stored in water for 24 h at 37°C and then underwent thermocycling. The SBS in megapascals (MPa) was determined by a shear test with 0.5 mm/min crosshead speed and failure types were classified with modified adhesive remnant index scores. The data were analyzed with one-way analyses of variance (ANOVA), Tukey and chi-square tests at the $\alpha = 0.05$ level. ***Results.*** Significant differences were found among the four groups ($F = 21.57$, $p < 0.01$). No significant difference was found between Group I and III (17.12 ± 2.84 and 15.08 ± 3.42 MPa, respectively) or between Group III and IV (12.82 ± 2.64 MPa). The lowest SBS value was determined in Group II (5.88 ± 2.12 MPa). Enamel demineralization, microabrasion therapy and CPP-ACP application affected enamel color significantly. ***Conclusion.*** CPP-ACP application and microabrasion therapy are able to increase the decreased SBS of orthodontic brackets because of enamel demineralization.

Key Words: *CPP-ACP, demineralization, microabrasion, shear bonding strength*

Introduction

Orthodontic appliances complicate oral hygiene and increase enamel demineralization; these are the most undesiable side-effects of orthodontic treatments [1–4]. Enamel demineralization is a sign of caries, which is a highly prevalent disease all around the world and involves molecular changes in the apatite crystals of the teeth [5]. In areas of demineralization light cannot pass in the dentin layer and, therefore, these lesions appear as milky white opacities [6,7].

The incidence of demineralized enamel surfaces is gradually increasing in the public. In other words, not only orthodontic treatment but also daily food habits can cause enamel demineralization [3,8]. Gorelick et al. [3] reported that 24% of patients who demanded orthodontic treatment had non-developmental enamel demineralization. More recently, Enia et al. [9] examined 400 orthodontically-treated patients and reported that 32.3% of patients had enamel demineralization before treatment and 73.5% had enamel demineralization after treatment.

When adequate amounts of calcium, phosphate and fluoride ions are provided, they can promote the remineralization of previously demineralized enamel surfaces [10]. In light of the contemporary orthodontic literature, a milk protein derivative, casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) has been recommended for caries prevention and enamel remineralization [11–13]. Daily use of CPP-ACP has been reported to induce remineralization by stabilizing the amount of calcium and phosphate ions present in the enamel. CPP-ACP has the ability to stabilize calcium and phosphate in an amorphous state, preventing the accumulation of calcium phosphate to critical levels, which leads to precipitation [14–16].

Microabrasion has become accepted as an effective, non-restorative, conservative treatment method to improve the appearance of teeth by removal of

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superficial non-carious enamel defects [17,18]. Akin and Basciftci [4] performed microabrasion on patients who had post-orthodontic enamel demineralization and showed that 99% of mild and moderate and 94% of severe enamel demineralization were sufficiently eliminated by microabrasion. It has been reported that as the removed minerals are plugged in the inter-prismatic area after microabrasion the enamel surface is less susceptible to bacterial colonization and demineralization than natural enamel [19].

It is believed that demineralized enamel surfaces are more permeable than natural enamel surfaces and can be easily affected by environmental conditions. Therefore, many researchers believe that by plugging the inter-prismatic pores susceptible enamel surfaces can be protected from environmental conditions [20]. Recently, resin infiltration was introduced to plug interprismatic pores and prevent lesions due to acid penetration.

In routine orthodontic practice, clinicians do not always match sound enamel surfaces. Also some functional, removable or bonded orthodontic appliances such as the twin-block appliances, rapid palatal expanders and bianotor can cause enamel demineralization [21]. It is important to understand which procedure or procedures will be successful for bonding of brackets when they are matched with demineralized enamel surfaces. Therefore, the aim of this study was to evaluate the effects of CPP-ACP application and microabrasion on the shear bond strength (SBS) of orthodontic brackets bonded to pre-treated demineralized enamel surfaces, as well as the effect of demineralization and inhibition of demineralization on enamel.

Materials and methods

This study was approved by the Regional Ethical Committee on Research of the Selcuk. University in Konya. The power analysis was established by G*Power version 3.0.10 software (Franz Faul Universitat, Kiel, Germany) software. Based on the 1:1 ratio between groups, a total sample size of 80 teeth was found to impart more than 80% power (actual power = 0.8453) in order to detect significant differences with a 0.40 effect size at the $\alpha = 0.05$ significance level.

Eighty non-carious, freshly extracted, sound human maxillary premolar teeth (from patients aged between 13–18 years) extracted with orthodontic indications were collected and placed in a solution of 0.1% thymol (for a maximum of 1 month). Teeth with hypoplastic areas, cracks, restoration or gross irregularities were excluded. The criteria for tooth selection dictated no pre-treatment with a chemical agent such as alcohol, formalin or hydrogen peroxide or any other form of bleaching. All residual tissue tags were cleaned from the tooth surface under running tap water. All teeth were mounted vertically in self-cure orthodontic acrylic until 2/3 of the root was embedded. The buccal surfaces of the teeth were cleaned and polished with oil and fluoride free fine pumice and water using a brush and a slow-speed handpiece, then rinsed with a water spray and dried with compressed air.

The teeth were randomly divided into four equal groups. Three of these groups were experimental and one of them was a control group.

- Group I: This group was the control group. No enamel demineralization procedure was performed in this group.
- Group II: Demineralization procedure was applied in this group. Brackets were directly bonded to the demineralized enamel surface.
- Group III: Demineralization procedure was performed in this group. CPP-ACP paste (MI Paste Plus, GC Europe, Leuven, Belgium) was then applied to the demineralized enamel surface for treatment of demineralized enamel. The paste was applied onto the enamel for 5 min and then rinsed with deionized water. Paste was re-applied after 6 h again and this procedure was then repeated 10-times. Between each application, all teeth were impacted in artificial saliva. The artificial saliva, which had an electrolyte composition similar to that of human saliva, was prepared from 0.103 g $CaCl_2H_2O$, 0.04 g $MgCl_26H_2O$, 0.544 g KH_2PO_4, 2 g N_3Na, 2.24 g KCl, 4.77 g Herpes Buffer and sufficient KOH to achieve pH 7.0 [22].
- Group IV: Demineralization was applied in this group and microabrasion therapy was then performed. Microabrasion agent (Opalstrue, Ultra-Dent, Utah, USA) was applied by an electronic toothbrush (Braun Oral-B Plaque Control 3D, Braun, Kronberg, Germany) for 3 min and then rinsed off with deionized water. This therapy was reapplied after 6 h and then repeated 5-times. Between each application, all teeth were impacted in artificial saliva, as in group III.

Demineralization procedure

Artificial sub-surface demineralized enamel surfaces were produced by immersion in demineralizing solution, a technique first described by Reynolds [23]. The enamel surfaces were exposed to demineralizing solution at 37°C for 3 weeks at pH 4.8. The solution composition was 40 mL of 0.1 mol/L lactic acid, 500 mg/L hydroxyapatite and 20 g/L Carbopol C907.

Bracket bonding procedure

Before bonding of brackets, the enamel surface was etched with 37% orthophosphoric acid (3M Dental Products, St Paul, MN) for 15 s. The etching liquid and demineralized tooth particles were removed with

an air–water syringe, which was applied for 10 s and teeth were then dried for 10 s with oil-free compressed air. After surface preparation, liquid Blue Glue primer (Ormco Corp., Glendora, CA) was applied to the etched surfaces and the brackets were bonded using Blue Glue light cure adhesive resin. Any excess adhesive resin around the brackets was removed using an explorer. A light-emitting-diode (LED) curing light (Elipar Freelight-2, 3M ESPE, Seefeld, Germany) was then applied for 20 s to cure the adhesive resin. Specimens in all groups were stored in distilled water at 37°C for 24 h and thermocycled for 10 000 cycles between 5 and 55°C, with a dwell time of 30 s at each temperature.

After the thermocycled procedure a knife-edge–shaped apparatus was placed between the joint of the enamel surface with the resin material. The SBS of the enamel was evaluated using a universal testing machine (TSTM 02500, Elista Ltd. Sti, Istanbul, Turkey) with a crosshead speed of 1 mm/min. The value of the maximum load required to debond the bracket was recorded in Newtons and converted to megapascals (1 MPa = 1 N/mm^2).

After SBS testing, all teeth and brackets were observed using a stereomicroscope at 40 × magnification (CX41, Olympus, Tokyo, Japan) to identify the mode of fracture. Any adhesive remaining after bracket removal was assessed using the adhesive remnant index (ARI) and scored according to the amount of resin adhering to the enamel surface.

Color measurements

Clinical spectrophotometer VITA Easyshade which comprises a base unit and a hand piece (VITA Zahnfabrik, Bad Säckingen, Germany) was used to measure color differences in each tooth according to the CIE L*a*b* color system. The spectrophotometer was automatically calibrated before each precision measurement and the probe was applied with an anti-infection cover for each tooth according to the manufacturer's instructions. All the measurements were performed keeping the tip of the spectrophotometer perpendicular and flush to the dried tooth surface and in contact with the tooth surface in a dark box.

Color was determined before and after the demineralization procedure in Group II and before and after experimental procedures in Groups III and IV. The quantitative ΔE values of groups were calculated with the following formula:

$$\Delta E = [(L^*2 - L^*1)2 + (a^*2 - a^*1)2 + (b^*2 - b^*1)2]1/2$$

Statistical analysis

All statistics were performed using SPSS version 17.0 (SPSS Inc, Chicago, IL). The Shapiro-Wilks test for normality and Levene's variance homogeneity test were applied to the data. For comparison of SBS the data were found to be normally distributed and there was homogeneity of variance among the groups. However, the data were not distributed normally in color measurement comparison. Thus, SBS measurements comparison were evaluated by using one-way analysis of variance (ANOVA) and post-hoc Tukey's multiple comparison test and in color measurement comparison intra-group comparisons were evaluated by using the Wilcoxon test and inter-group changes were analyzed using the Kruskal-Wallis test and post-hoc comparisons were done by the Mann-Whitney *U*-test at $p <. 05$.

For the ARI scores, the chi-square test was used to identify any significant differences among the groups.

Results

The descriptive statistics, including mean values, standard deviations, minimum and maximum values and statistical comparison of the groups are presented in Table I. According to ANOVA, there were significant differences between the SBS values of the groups ($F = 21.57$, $p < 0.01$). The highest and lowest SBS values were found in Groups I and II, respectively (Group I = 17.12 ± 2.84 MPa and Group II = 5.88 ± 2.12 MPa). There were no significant differences between Groups I and III ($p > 0.05$) (Group III = 15.08 ± 3.42 MPa) or between Groups III and IV ($p > 0.05$) (Group IV = 12.82 ± 2.64 MPa). Significant differences were found between Groups I and II ($p < 0.001$), Groups I and IV ($p < 0.05$) and Groups III, IV and II ($p < 0.01$).

To assess the amount of resin left on the enamel surfaces after debonding, the ARI score was used. The ARI scores for the various groups tested are listed in Table II. The chi-square comparison test indicated that there were significant differences among the four groups ($\chi^2 = 59.005$, $p < 0.001$). Enamel detachment was seen in Group II.

The descriptive statistics and statistical comparison (before and after experimental procedures) of the L, a and b color measurement values are presented in Table III. Enamel demineralization decreased L and increased a and b values. CPP-ACP application and microabrasion therapy increased L and decreased a and b values of the tooth.

Mean differences and post-hoc statistical comparisons of the ΔL, Δa, Δb and ΔE color measurement values of groups are presented in Table IV. There were significant differences between the ΔL, Δa, Δb and ΔE values of the groups. The comparison of ΔE showed that color changes could be seen visually in all groups, but changes were more obvious in the demineralization and microabrasion groups.

Table I. Descriptive statistics and the results of ANOVA and Tukey's comparing SBS.

					Sign	
Groups	*n*	Mean	SD	Min–Max	ANOVA	Tukey
Control	20	17.12	2.84	13.64–23.76	$p < 0.01$	A
Demineralization	20	5.88	2.12	3.12–9.56		B
CPP-ACP	20	15.08	3.42	11.05–21.67	$F = 21.57$	AC
Microabrasion	20	12.82	2.64	9.75–16.54		C

Discussion

In this study we aimed to determine which demineralization inhibition method is suitable for SBS of orthodontic brackets and color effects of these methods. The results of the present study showed that both of demineralization inhibition methods improved SBS of orthodontic brackets, but CPP-ACP application was better than microabrasion therapy. The maximum mean SBS was obtained in the control group. The SBS values of the CPP-ACP group and microabrasion group followed this. The mean SBS value of the control group was significantly higher than that of the microabrasion group. However, no significant differences in mean SBS value were found between the control and CPP-ACP groups or the CPP-ACP and microabrasion groups. On the other hand, demineralization and both demineralization inhibition methods affected enamel color significantly.

Artificially demineralized lesions and naturally demineralized lesions are not identical, but they are quite similar. Thus, there are advantages of using artificially demineralized lesions when performing mechanistic studies [24,25]. Therefore, we prepared artificially demineralized lesions in the present study, using a technique first described by Reynolds. Demineralization of the enamel surface decreased the SBS of orthodontic brackets, as shown in many previous studies [21,25,26]. Uysal et al. [25] attributed this result to the poor quality of the enamel surface and a lack of resin tags for the formation of mechanical interlocking.

In the contemporary orthodontic literature, CPP-ACP applications have been accepted as a means by which to provide remineralization potential to previously demineralized enamel. Additionally, increased levels of calcium and phosphate ions in supragingival plaque caused by CPP-ACP application have been demonstrated. Although the potential of CPP-ACP to promote remineralization was demonstrated in animal caries models in 1995, it was used for the first time in treating enamel demineralization in 2009 by Bailey et al. [27].

In removing of superfacial non-carious enamel defects microabrasion has long been widely used and this conservative technique has also been used to remove white spot lesions [17,18,28]. In a recent study [4], effects of CPP-ACP application and microabrasion on treatment of white spot lesions was compared with a control group. It was found that microabrasion was an effective method for the treatment of white spot lesions and followed CPP-ACP with regards to effectiveness.

Keçik et al. [29] found that CPP-ACP significantly increased the mean SBS values of orthodontic brackets; in contrast Tabrizi and Cakırer [30] found no significant differences when CPP-ACP was compared with a control group. Natural enamel surfaces were used in these studies. On the other hand, Baysal and Uysal [21] and Uysal et al. [25] found that pretreatment of artificially demineralized surfaces with CPP-ACP appeared to restore decreased SBS values of orthodontic brackets. The results of the present study are in accordance with these studies.

Initially, every clinician is wary of microabrasion as it can lead to the removal of too much enamel material [4]. However, Waggoner et al. [31] reported an average removal of 12 μm of enamel material after initial

Table II. Frequency of distributions and comparison of ARI scores.

Groups	*n*	1	2	3	4	5
Control[a]	20	12 (60%)	6 (30%)	2 (10%)	0	0
Demineralization[b]	20	0	0	2 (10%)	6 (30%)	12 (60%)
CPP-ACP[c]	20	8 (40%)	6 (30%)	4 (20%)	2 (10%)	0
Microabrasion[c]	20	6 (30%)	5 (25%)	6 (30%)	3 (15%)	0

ARI scores, 1: all of composite, with impression of bracket base, remained on tooth; 2: more than 90% of composite remained; 3: more than 10% but less than 90% of composite remained on tooth; 4: less than 10% of composite remained on tooth surface; 5: no composite remained on enamel.
$p > 0.001$, $\chi^2 = 59.005$.

Table III. Descriptive statistics and intra-group comparison of L*, a*, b* values.

Groups	*n*	L1 Mean	L1 Min–max	L2 Mean	L2 Min–max	Sign
Demineralized	20	83.01 ± 1.68	79.50–85.40	78.40 ± 3.98	71.50–84.90	**
CPP-ACP	20	78.18 ± 2.84	74.80–83.20	82.46 ± 2.16	78.20–84.70	***
Microabrasion	20	77.02 ± 3.10	71.80–81.30	86.87 ± 2.37	83.00–90.00	***
		a1		a2		
Demineralized	20	0.02 ± 0.88	–1.31–2.21	2.25 ± 1.00	0.90–4.00	***
CPP-ACP	20	1.66 ± 0.90	0.10–3.20	1.29 ± 0.83	–0.41–2.39	**
Microabrasion	20	1.67 ± 0.88	0.20–3.00	0.10 ± 0.54	–0.91–0.81	***
		b1		b2		
Demineralized	20	25.89 ± 2.99	19.70–30.60	32.01 ± 2.11	29.60–35.60	***
CPP-ACP	20	31.26 ± 3.12	26.70–36.30	29.99 ± 2.68	25.10–33.70	**
Microabrasion	20	30.26 ± 3.26	23.00–34.20	27.58 ± 2.82	22.30–32.00	***

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

application and an average of 26 μm of enamel loss after each successive application. We determined that microabrasion therapy partially restored the decreased SBS values of orthodontic brackets after demineralization. There was a significant difference in mean SBS values between the microabrasion and control groups. This may be attributed to the fact that the microabrasion process compacts removed minerals in the prismatic area. The result of the present study is in accordance with the study by Baysal and Uysal [21]. On the other hand, this result is not in agreement with the study by Sanders et al. [32], which suggested that there were no significant differences in the SBS values of orthodontic brackets between microabraded and non-microabraded enamel surfaces.

To our knowledge there is only one study [33] on the effect of methods for inhibition of demineralization on enamel color. Paic et al. [33] investigated the effect of microabrasion therapy on enamel surface color; they suggested that microabrasion therapy did not affect enamel color significantly. In this study microabrasion was performed on the natural enamel surface and only applied for 40 s. The results of the present study did not support the results of Paic et al. [33]. The ΔL* co-ordinate represents the brightness of an object represented on the *y*-axis, the Δa* value represents the red (positive *x*-axis) or green (negative *x*-axis) chroma and the Δb* value represents the yellow (positive *z*-axis) or blue (negative *z*-axis) chroma. The color difference (ΔE) of two objects can be determined by comparing the differences between respective co-ordinate values for each object [34,35]. In the present study, ΔE* was calculated from the formula mentioned in the Materials and methods section. The ΔE* value has been used to evaluate the 'perceptibility' of color differences by many authors [36–38]. However, it is noteworthy that the criteria of perceptibility adopted by each author were different. While color changes of less than 1.0 ΔE* units were not seen visually, those between 1.0–3.3 were deemed to be clinically acceptable [39,40]. The results of the present study showed that enamel demineralization, CPP-ACP application and microabrasion affected enamel color significantly. The mean ΔE* values of the groups were not within an acceptable limit. Enamel demineralization decreased the brightness of enamel and made the color of enamel redder and more yellow. In contrast, CPP-ACP application and microabrasion therapy increased the brightness of enamel and made the color of enamel more green and blue.

Table IV. Descriptive statistics and inter-group comparison of mean ΔL, Δa, Δb, ΔE values.

Groups	*n*	Mean	SD	Sign ($p < 0.001$)
ΔL				
Demineralized	20	−4.61	4.71	A
CPP-ACP	20	4.28	1.92	B
Microabrasion	20	9.85	2.29	C
Δa				
Demineralized	20	2.23	1.37	A
CPP-ACP	20	−0.37	0.46	B
Microabrasion	20	−1.57	0.65	C
Δb				
Demineralized	20	5.62	3.35	A
CPP-ACP	20	−1.27	1.50	B
Microabrasion	20	−2.68	1.31	B
ΔE				
Demineralized	20	8.38	4.71	A
CPP-ACP	20	4.72	1.96	B
Microabrasion	20	10.38	2.50	A

Reynolds [41] suggested that the minimum bond strength value that is adequate for most clinical orthodontic needs and routine clinical use is 5.9–7.8 MPa. In the current study, all groups except for the demineralization group (5.88 ± 2.12 MPa) exhibited greater SBS values than this, which indicates that the SBS values of the control, CPP-ACP and microabrasion groups were sufficient for clinical use. However, the suggested values are based on tensile strength, whereas SBS values were evaluated in the present study.

The results of ARI score comparisons in the present study indicated that there were significant differences among the four groups tested. According to the ARI scores, no detachment was found at the enamel–composite interface in the control, CPP-ACP and microabrasion groups, whereas in the demineralization group, more than half (60%) of the total detachments occurred between the enamel–composite interfaces.

Although it is impossible to create laboratory conditions that fully represent the oral environment, every effort and thermocycled procedure were made to standardize the testing procedure in an attempt to create a laboratory technique which was as representative of the clinical situation as possible. It is true that *in vitro* bond strength testing is not fully representative of intra-oral conditions. However, it can give an idea of the clinical performance of the various groups tested.

Conclusions

Enamel demineralization significantly reduces the SBS of orthodontic brackets and changes enamel color, which can be detected visually.

CPP-ACP application and microabrasion procedures restore the decreased SBS of orthodontic brackets and change enamel color caused by enamel demineralization.

CPP-ACP is more efficient than microabrasion for restoring decreased SBS values.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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