Original Article

Effects of remineralization procedures on shear bond strengths of brackets bonded to demineralized enamel surfaces with self-etch systems

Zeliha Müge Baka[a]; Mehmet Akin[a]; Zehra Ileri[a]; Faruk Ayhan Basciftci[b]

ABSTRACT

Objective: To compare the effects of different remineralization procedures on the surface roughness of teeth, shear bond strengths (SBSs), and Adhesive Remnant Index scores of self-etching primer (SEP) used to bond orthodontic brackets to previously treated demineralized enamel surfaces.

Materials and Methods: A total of 140 extracted human premolar teeth were randomly divided into seven equal groups. Group I was the control group. A demineralization procedure was performed in the other six groups. A remineralization procedure was performed before bonding by using casein phosphopeptide-amorphous calcium phosphate, fluoride, a microabrasion mixture (18% hydrochloric acid-fine pumice), a microabrasion agent, and resin infiltration in groups III to VII. Brackets were bonded using a self-etching primer/adhesive system. The specimens were tested for SBS. The roughness and morphology of the enamel surfaces were analyzed using profilometer and scanning electron microscopy. Data were analyzed with analysis of variance, Tukey, and G-tests at the $\alpha = .05$ level.

Results: Significant differences were found in the SBS values among the seven groups ($F = 32.69$, $P = .003$). The lowest SBS value was found in group II (2.62 $\pm$ 1.46 MPa). No significant differences were found between groups I, III, and VII, between groups III and IV, or between groups V and VI. The differences in the roughness values were statistically significant among the groups ($P = .002$).

Conclusions: Remineralization procedures restore the decreased SBS of orthodontic brackets and decrease surface roughness caused by enamel demineralization. SEPs provide clinically acceptable SBS values for bonding orthodontic brackets to previously treated demineralized enamel surfaces. (*Angle Orthod.* 2016;86:661–667.)

KEY WORDS: Enamel; Remineralization; Demineralization; Self-etch system

INTRODUCTION

Orthodontic appliances lead to the accumulation of dental plaque by creating new retention areas. They also complicate oral hygiene procedures and increase the risk of formations of white spot lesions (WSLs).[1] However, WSLs can also be seen in orthodontically untreated individuals. Gorelick et al.[2] found that the incidence of white spot formation in patients treated with fixed orthodontic appliances was nearly 50% compared to 24% in an untreated control group. Recently, Lucchese and Gherlone[3] found WSLs in 13% of orthodontically untreated patients. Furthermore, some orthodontic appliances (functional appliances, rapid maxillary expansion appliances, etc.) can lead to the formation of WSLs during the initial phase of orthodontic treatment.[4] Therefore, the clinician may need to give additional attention to the bonding phase of orthodontic brackets in such patients and could help these patients by improving the WSLs with several remineralization procedures before fixed orthodontic treatment.

Overall, different remineralization procedures have been recommended for the management of WSLs. WSLs can be managed with fluoride and casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) applications, enamel microabrasion, and

[a] Assistant Professor, Department of Orthodontics, Faculty of Dentistry, Selcuk University, Konya, Turkey.

[b] Professor and Chair, Department of Orthodontics, Faculty of Dentistry, Selcuk University, Konya, Turkey.

Corresponding author: Dr Zehra Ileri, Assistant Professor, Selçuk Üniversitesi, Dişhekimliği Fakültesi, Ortodonti AD, Selçuklu 42079, Kampüs/Konya, Turkey
(e-mail: zehra.ileri@gmail.com)

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bleaching.[5] Fluoride provides remineralization of early enamel lesions and slows the progress of the carious process by forming flourapatite.[6] If adequate amounts of salivary or plaque calcium and phosphate ions are available, the remineralization of previously demineralized enamel can be promoted by fluoride ions.[7] CPP-ACP, which is derived from milk casein, can also be used for remineralization. It has been shown that CPP-ACP increases the levels of calcium and phosphate ions significantly in supragingival plaque and promotes the remineralization of enamel subsurface lesions in situ.[8] Another remineralization procedure is microabrasion, and it has been extensively used to remove superficial noncarious enamel defects.[9] Microabrasion abrades the enamel surface and leaves a highly polished surface with calcium phosphate packed into the interprismatic area. This surface is less susceptible to bacterial colonization and demineralization than natural enamel.[10] Recently, a new approach called *resin infiltration* has been used for noncavitated lesions. In this method, the pore system of a noncavitated white spot is filled or reinforced with a light-curable resin.[11]

In orthodontic practice, bonding orthodontic brackets to an enamel surface that has undergone a remineralization procedure may be required. It is well known that it is important to ensure a reliable connection between the enamel and the orthodontic bracket. Previous studies were conducted with conventional bonding methods and reported that remineralization procedures improved the bonding to demineralized enamel.[12–14] Self-etching primers (SEPs) were introduced to improve the bonding procedures. SEPs have some advantages, such as reduced loss of enamel, prevention of saliva contamination, and less chair time when compared to conventional methods. In addition, SEPs showed promising adhesive bonding results.[15]

To our knowledge, no study has evaluated the efficiency of SEPs when used for orthodontic bonding on previously treated demineralized enamel. Therefore, the aim of this study was to compare the effects of different remineralization procedures on the surface roughness of teeth, shear bond strengths (SBSs), and Adhesive Remnant Index (ARI) scores of SEPs used to bond orthodontic brackets to previously treated demineralized enamel surfaces.

MATERIALS AND METHODS

Ethical approval for this study was obtained from the Regional Ethical Committee on Research of the Selçuk University in Konya, Turkey. A power analysis was performed by G*Power version 3.0.10 software (Franz Faul Universitat, Kiel, Germany). Based on the 1:1 ratio between groups, a total sample size of 140 teeth was found to grant more than 85% power (actual power = 0.8777) to detect significant differences with a 0.35 effect size at the $\alpha = .05$ significance level.

A total of 140 noncarious human maxillary premolar teeth freshly extracted with orthodontic indications were collected and put into a 0.1% thymol solution for a maximum of 1 month. Teeth with hypoplastic areas, cracks, restorations, or gross irregularities were excluded. The criteria for tooth selection dictated no pretreatment with a chemical agent (alcohol, formalin, or hydrogen peroxide). All residual tissue tags were cleaned from the teeth. All teeth were mounted vertically in self-cure orthodontic acrylic blocks until two-thirds 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 water and dried.

The teeth were randomly divided into seven equal groups of 20 teeth. Six of these groups were experimental and one of them was a control group. In the experimental groups, all teeth were demineralized and then remineralization procedures (described in Table 1) were applied to five groups except group II.

Between each application, all teeth were stored in artificial saliva. The artificial saliva, which had an electrolyte composition similar to that of human saliva, was prepared from 0.103g $CaCl_2H_2O$, 0.04g $MgCl_26H_2O$, 0.544g KH_2PO_4, 2g N_3Na, 2.24g KCl, 4.77g Herpes Buffer, and sufficient potassium hydroxide (KOH) to achieve a pH of 7.0.[16]

Artificial subsurface demineralized enamel surfaces were created by immersion in demineralizing solution, a technique first described by Reynolds.[17] 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. The presence of demineralization was identified with a laser fluorescence device (DIAGNOdentPen, KaVo, Biberach, Germany) before and after the demineralization procedure. The enamel caries threshold of >13 was ensured in all of the demineralized teeth used in this study.

At the bonding phase of orthodontic brackets followed by pretreatments, Transbond Plus Self-Etching Primer (3M Unitek, Monrovia, Calif) containing both the etching agent and the primer was rubbed onto the enamel surface for approximately 3 seconds. An oil and moisture-free air source was then used to deliver a gentle air burst to the tooth. Orthodontic metal brackets (0.018 inch slot; Roth-equilibrium® 2, 722-341, Dentaurum, Pforzheim, Germany) were then bonded with Transbond XT (3M Unitek) light-cure

Table 1. Description of the Remineralization Procedures

Group	Pretreatment	Description of Remineralization Procedure	Manufacturer
Group I	No (control)		
Group II	Demineralization		
Group III	Demineralization + CPP-ACP[a]	CPP-ACP paste was applied to the demineralized enamel surface before bonding. The paste was applied onto the enamel for 5 min and then rinsed with deionized water. It was reapplied after 6 h, and this procedure was repeated 10 times, as stated in the study of Uysal et al.[12]	GC Tooth Mousse, Asia Pty. Ltd, Tokyo, Japan
Group IV	Demineralization + Fluoride	Fluoride gel was applied to the demineralized enamel surface before bonding. The gel was applied according to the same protocol as described for group III.	Bifluorid 12; Voco-GmbH, Cuxhaven, Germany
Group V	Demineralization + Microabrasion 1	Microabrasion therapy was applied to the demineralized enamel surface before bonding. A mixture was prepared with 18% hydrochloric acid and fine pumice powder, which is described by Welbury and Carter.[27] The mixture was applied for 3 min and then rinsed off with deionized water. This therapy was reapplied after 6 h and repeated 5 times.	Handmade
Group VI	Demineralization + Microabrasion 2	Microabrasion agent was applied to the demineralized enamel surface before bonding. It is a 6.6% hydrochloric acid (HCl) slurry that contains silicon carbide microparticles. The agent was applied according to the same protocol as described for group V.	Opalstrue, UltraDent, South Jordan, Utah, USA
Group VII	Demineralization + Resin Infiltration	A resin infiltrant was applied to the demineralized enamel surface according to the manufacturer's recommendations before bonding. Preconditioning with the resin infiltrant included hydrochloric acid etching (15% HCl, 2 min; Icon-Etch), water rinsing (30 s), surface drying by ethanol (30 s, Icon-Dry), application of a low-viscosity resin infiltrant (3 min, Icon-Infiltrant), and light-curing (with an intensity of 1200 mW/cm^2) for 40 s. The etching step was performed only once in all teeth treated with resin infiltrant.	ICON®; DMG, Hamburg, Germany

[a] CPP-ACP indicates casein phosphopeptide-amorphous calcium phosphate.

adhesive resin. Any excess adhesive resin around the brackets was removed using an explorer. A light-emitting-diode curing light (Elipar S10, 3M ESPE, Seefeld, Germany) was then applied from the mesial and distal directions for 20 seconds. All specimens were stored in distilled water at 37°C for 24 hours and thermocycled for 5000 cycles between 5°C and 55°C, with a dwell time of 30 seconds at each temperature.

After the thermocycled procedure, a knife edge–shaped apparatus was placed at the enamel-resin interface. 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 the debonding procedure, all teeth and brackets were observed using a stereomicroscope (CX41, Olympus, Tokyo, Japan) at 40× magnification to identify the type of fracture. Any adhesive remaining after bracket removal was assessed using the ARI and scored according to the amount of resin adhering to the enamel surface.

The surface roughness of the teeth was assessed using a profilometer (Mitotoyo Surf Test SJ 201 P/M, Mitutoyo Corp., Takatsu-ku, Japan) at the baseline and after the remineralization procedures. To measure the roughness profile value in micrometers, a diamond stylus (tip radius, 5μm) was moved across the surface under a constant load of 0.75 mN with a speed of 0.5 mm/sec and a range of 350 μm. The instrument was calibrated using a standard precision reference specimen. Average roughness (Ra) was recorded for each specimen.

Furthermore, enamel surface morphology (smooth, demineralized, and treated with remineralization procedures) was observed by scanning electron microscopy (SEM; LEO 435 VP, LEO Elektronenmikroskopie GmbH, Oberkochen, Germany).

The Shapiro-Wilks test for normality and Levene's test for variance homogeneity were applied to the data. For comparison of SBS and surface roughness, the data were found to be normally distributed, and there was homogeneity of variance among the groups. Thus, SBS and surface roughness measurement comparisons were evaluated using one-way analysis of variance (ANOVA) and post hoc Tukey's multiple comparison tests at $P < .05$. For the ARI scores, the G-test was used to identify any significant differences among the groups.

Two weeks after the first measurements, 20 teeth before the remineralization procedure and 20 teeth after the remineralization procedure were randomly selected and Ra values were remeasured by the same operator. Correlation analysis yielded an r value of 0.968. The method error was calculated using Dahlberg's formula and the value was 0.921 within acceptable limits.

RESULTS

The descriptive statistics and statistical comparisons of the SBS values for seven groups are presented in

Table 2. The Descriptive Statistics (in MPa) and Statistical Comparison of the Shear Bond Strength Values

Group	n[a]	Remineralization Procedure	Mean	SD[a]	Min–Max[a]	SIGN[a] ANOVA[a]	SIGN[a] TUKEY
Group I	20	No (control)	10.21	2.26	7.16–13.62		A
Group II	20	Demineralization	2.62	1.46	0–5.36	$P = .003$	B
Group III	20	CPP-ACP[a]	9.04	2.64	5.92–12.54		AC
Group IV	20	Flouride	7.92	2.12	4.41–11.62		C
Group V	20	Microabrasion 1	6.18	1.65	4.04–9.17		D
Group VI	20	Microabrasion 2	6.54	1.83	4.54–9.04	$F = 32.69$	D
Group VII	20	Resin infiltration	10.06	2.08	7.12–13.58		A

[a] n indicates sample size; SD, standard deviation; Min, minimum; Max, maximum; SIGN, significance; ANOVA, analysis of variance; CPP-ACP, casein phosphopeptide-amorphous calcium phosphate.

Table 2. According to the ANOVA, there were significant differences between the SBS values of the groups ($F = 32.69$, $P = .003$). The highest and lowest SBS values were found in groups I and II, respectively (group I = 10.21 MPa, group II = 2.62 MPa). There were no significant differences between groups I, III, and VII (group III = 9.04 MPa, group VII = 10.06 MPa), between groups III and IV (group IV = 7.92 MPa), or between groups V and VI (group V = 6.18 MPa, group VI = 6.54 MPa).

The ARI scores for the groups tested are listed in Table 3. In general, enamel detachment was seen in the groups. The G-test indicated that there were no significant differences among the seven groups ($P = .108$).

The descriptive statistics and statistical comparison of the surface roughness measurements are presented in Table 4. Statistical analysis showed that there were significant differences between the surface roughness values of the groups ($P = .002$). The demineralization group (group II) showed higher roughness when compared with the control group (group I). The microabrasion groups (group V and group VI) showed lower roughness when compared with the demineralization group (group II). There were no significant differences between group III and group IV, or between group V and group VI. The SEM images of the smooth, demineralized, and treated enamel surfaces are presented in Figure 1. SEM analysis corroborated the measurements for surface roughness. The teeth treated with microabrasion procedures caused the most smooth enamel surfaces.

DISCUSSION

In this study, artificially demineralized lesions were created and then different remineralization procedures were performed to determine which procedure was most appropriate for the SBS of orthodontic brackets and the surface roughness of teeth. Results of this study showed that all of the remineralization procedures used in this study improved the SBS of orthodontic brackets bonded to demineralized enamel surfaces and reduced the surface roughness of demineralized teeth. The highest mean SBS value was obtained in the control group. This was followed by resin infiltration and CPP-ACP groups, and there were no significant differences between control, resin infiltration, and CPP-ACP groups. The mean SBS value of the control group was significantly higher than that of the microabrasion-1, microabrasion-2, and fluoride groups. However, no significant differences in mean SBS value were found between the fluoride and CPP-ACP groups.

Table 3. Frequency of Distributions and Comparison of Adhesive Remnant Index (ARI) Scores

Group	n[a]	Remineralization Procedure	ARI Scores[b] 0	1	2	3	Significance (G-Test)
Group I	20	Control	10	6	3	1	
Group II	20	Demineralization	18	2	0	0	
Group III	20	CPP-ACP[a]	14	4	2	0	
Group IV	20	Fluoride	15	4	1	0	$P = .108$
Group V	20	Microabrasion 1	16	3	1	0	
Group VI	20	Microabrasion 2	16	4	0	0	
Group VII	20	Resin infiltration	12	6	2	0	

[a] n indicates sample size; CPP-ACP, casein phosphopeptide-amorphous calcium phosphate.

[b] 0, no adhesive remaining on the enamel surface; 1, less than 50% adhesive remaining on enamel surface; 2, more than 50% adhesive remaining on enamel surface; 3, all adhesive remaining on enamel surface.

Table 4. The Descriptive Statistics (in μm) and Statistical Comparison of the Surface Roughness Measurements

	Ra[a] (Mean Control Ra = 0.74)				
Group	Mean	SD[a]	Min–Max[a]	ANOVA[a]	TUKEY
Control–Demineralization	(–)0.86	0.12	0.63–1.15	$P = .002$	A
Demineralization–CPP-ACP[a]	0.62	0.08	0.46–0.78		B
Demineralization–Fluoride	0.57	0.10	0.49–0.83		B
Demineralization–Microabrasion 1	1.32	0.15	1.07–1.64	$F = 46.12$	C
Demineralization–Microabrasion 2	1.28	0.17	1.02–1.62		C
Demineralization–Resin infiltration	0.82	0.13	0.61–0.98		D

[a] Ra indicates average roughness; SD, standard deviation; Min, minimum; Max, maximum; ANOVA, analysis of variance; CPP-ACP, casein phosphopeptide-amorphous calcium phosphate.

Keçik et al.[18] compared the effect of acidulated phosphate fluoride (APF) and CPP-ACP on the SBS of orthodontic brackets and found that APF and CPP-ACP significantly increased the mean SBS values of orthodontic brackets; in contrast, Tabrizi and Cakirer[19] found no significant differences in the CPP-ACP group and also found significant decreases in the APF group when compared with a control group. In the studies mentioned above, natural enamel surfaces were used. On the other hand, Uysal et al.[12] compared the effects of fluoride and CPP-ACP and Baysal and Uysal[13] compared the effects of microabrasion and CPP-ACP on the SBS of orthodontic brackets bonded to demineralized enamel and found no significant differences between the control and CPP-ACP groups. In both studies, pretreatment of artificially demineralized surfaces with remineralization procedures appeared to restore decreased SBS values of orthodontic brackets, which is in accordance with the findings of our study. In contrast to our study, which showed significant increases in the SBS of orthodontic brackets after fluoride treatment when compared with the demineralization group, Ekizer et al.[14] found no significant differences between fluoride and demineralization groups.

The prevention of caries formation or progression by resin infiltration and penetration of adhesives into previously demineralized enamel seem to be promising approaches for the nonrestorative treatment of enamel lesions.[11] In this study, the resin infiltration group showed higher SBS values when compared with other remineralization methods in agreement with the findings of Ekizer et al.[14] They attributed this result to the convenience of the resin penetration into demineralized enamel, which results in micromechanical interdigitation strengthening.

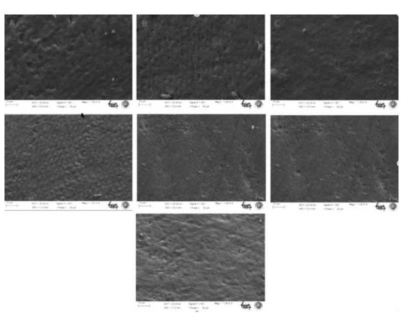

Figure 1. The scanning electron microscopy images of smooth enamel (A), demineralized enamel (B), and enamel treated with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) (C), fluoride (D), microabrasion 1 (E), microabrasion 2 (F), and resin infiltration (G).

Microabrasion is an effective treatment method for improving demineralized enamel lesions.[20] This method improved the SBS values of orthodontic brackets bonded to demineralized enamel. The mean SBS value of the control group was significantly higher than that of the microabrasion-1 and microabrasion-2 groups. There was no significant difference in mean SBS values between the two microabrasion groups. This result is in accordance with the study by Baysal and Uysal.[13] On the other hand, in contrast to our study, Sanders et al.[21] indicated similar SBS values of orthodontic brackets bonded to microabraded and nonmicroabraded tooth surfaces. The different results may be due to the differences in enamel conditions and abrasives used.

According to Reynolds,[22] 5.9–7.8 MPa SBS values are adequate for most clinical orthodontic needs. In this study, all groups except the demineralization group (2.62 MPa) showed greater SBS values than the values suggested by Reynolds. The SBS values of the control, CPP-ACP, fluoride, microabrasion-1, microabrasion-2, and resin infiltration groups were sufficient for clinical use. Therefore, application of any remineralization procedures for improving the WSLs before fixed orthodontic treatment can be considered.

According to the ARI scores, there was no significant difference among the seven groups tested. In general, enamel detachment was seen in all groups. Considering the location of separation, all groups showed a higher prevalence of ARI scores of 0 and 1, meaning that the bond between the bracket and resin was stronger than that between the resin and enamel. These results can be associated with using SEP in the present study. In routine orthodontic practice, it is important to achieve a reliable bonding between orthodontic attachments and tooth surfaces. Generally, 37% phosphoric acid application increases the bond strength of orthodontic brackets.[23] Despite increasing the durability of resin attachment, one of its potential disadvantages is the demineralization of the most superficial layer, which makes the enamel surface more susceptible to long-term acid attack and caries.[24] Therefore, in this study SEP was used for improving bonding procedures. Although enamel detachment was seen in the groups, SEPs provide us with clinically acceptable SBS values except for the demineralization group. Demineralization of teeth decreases the quality of bonding as stated by Uysal et al.[12] and Ekizer et al.[14]

The surface roughness of teeth and restoration correlates directly with the retention of plaque because bacteria adhere more readily to rough surfaces. The mean critical value of surface roughness for bacterial colonization has been defined as 0.2 μm.[25] In the present study, all mean surface roughness values were above this critical value, especially in the demineralization group. Remineralization procedures reduced the surface roughness of demineralized teeth. This effect is highly recommended for preventing bacterial colonization before fixed orthodontic treatment in caries-risk patients.

Thermocycling aims to thermally stress the adhesive joint interface; 3000 thermocycles between 5°C and 55°C is suggested to equal the number of years of intraoral thermocycling, exceeding the average orthodontic treatment term.[26] Therefore, we performed the thermocycling procedure for 5000 cycles to represent the clinical situation as closely as possible. Although the laboratory conditions do not fully reflect the oral environment, it provides an idea of the clinical performance of the various groups tested.

CONCLUSIONS

- Enamel demineralization significantly reduces the SBS of orthodontic brackets and increases surface roughness. CPP-ACP and fluoride applications, microabrasion procedures, and resin infiltration restore the decreased SBS of orthodontic brackets and decrease surface roughness caused by enamel demineralization.
- Resin infiltration and CPP-ACP is more efficient than other groups for restoring decreased SBS values.
- Microabrasion is more efficient for decreasing surface roughness caused by enamel demineralization.
- SEPs provide clinically acceptable SBS values for the bonding of orthodontic brackets to previously treated demineralized enamel surfaces.

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