Acta Odontologica Scandinavica. 2016; 74: 7–13

informa healthcare

ORIGINAL ARTICLE

Effects of different pre-treatment methods on the shear bond strength of orthodontic brackets to demineralized enamel

ILKNUR VELİ[1], MEHMET AKIN[2], ZELİHA MÜGE BAKA[2] & TANCAN UYSAL[1]

[1]*Department of Orthodontics, Faculty of Dentistry, Izmir Katip Celebi University, Izmir, Turkey, and*
[2]*Department of Orthodontics, Faculty of Dentistry, Selcuk University, Konya, Turkey*

Abstract

Objective. To compare the effects of different treatment methods used for the enamel damage, on the shear bond strength (SBS) and fracture mode of orthodontic brackets. ***Materials and methods.*** Freshly-extracted 140 premolars were randomly allocated to seven groups: Group I was considered as the control of other groups. The remaining groups were exposed to demineralization. In group II, brackets were directly bonded to the demineralized enamel surface. CPP-ACP paste (GC Tooth Mousse), fluoride varnish (Bifluorid 12), microabrasion with a mixture prepared with 18% hydrochloric acid and fine pumice powder, microabrasion with an agent (Opalustre) and resin infiltrant (Icon®) were applied in Groups III, IV, V, VI and VII, respectively. The specimens were tested for SBS and bond failures were scored according to the Adhesive Remnant Index (ARI). Analysis of variance and Tukey tests were used to compare the SBS of the groups. ARI scores were compared with G-test. The statistical significance was set at $p < 0.05$ level. ***Results.*** Statistically significant differences were found among seven groups ($F = 191.697$; $p < 0.001$). The SBSs of groups I (mean = 18.8 ± 2.0 MPa) and VII (mean = 19.1 ± 1.4 MPa) were significantly higher than the other groups. No statistically significant difference was found between groups IV (mean = 11.5 ± 1.2 MPa) and V (mean = 12.6 ± 1.5 MPa). The differences in ARI scores of the groups were statistically significant ($p < 0.01$). ***Conclusions.*** All demineralization treatment methods improve bonding to demineralized enamel. Resin infiltrant application after demineralization showed similar bond strength values as intact enamel.

Key Words: *CPP-ACP, fluorides, enamel microabrasion, shear strength*

Introduction

Enamel demineralization is an early stage of dental caries that occurs when plaque is allowed to remain on the tooth surface for a critical length of time [1,2]. Demineralization is a side-effect of orthodontic treatment, with rates reported between 2–96% [3], and the existence of fixed appliances has been associated with prolonged accumulation of bacterial plaque on the enamel surfaces [4]. Clinically, an early lesion is an opaque white area that reflects a marked loss of minerals below the outermost enamel layer. The tooth surface at this stage is intact, although forceful probing can cause cavitation [5]. In order to prevent enamel demineralization, orthodontists have tried different techniques and materials [6,7].

Studies show that high oral hygiene and local use of fluoride with low concentration are the primary solutions for minimizing demineralization around the brackets [8,9]. Fluoride ions can be incorporated into the hydroxyapatite structure of tooth enamel by the redeposition of dissolved hydroxyapatite as less soluble fluoridated species [10]. There are several methods of delivering fluoride to teeth in patients during orthodontic treatment. These include topical fluorides (e.g. mouthrinse, gel, varnish, toothpaste) and fluoride-releasing materials (e.g. bonding materials, elastics) [11]. Although the orthodontic specialty has focused mainly on protocols for fluoride intervention, other mechanisms such as casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) applications, enamel microabrasion and resin infiltrants have been suggested as mechanisms to inhibit demineralization.

Correspondence: Professor Dr Tancan Uysal, İzmir Katip Çelebi Üniversitesi, Diş Hekimliği Fakültesi, Ortodonti A.D, Aydınlıkevler Mah, Çiğli, 35640, İzmir, Turkey. Tel: +90 2323254040 2205. Fax: +90 2323252535. E-mail: tancanuysal@yahoo.com

(Received 20 April 2014; accepted 22 October 2014)

ISSN 0001-6357 print/ISSN 1502-3850 online © 2015 Informa Healthcare
DOI: 10.3109/00016357.2014.982703

CPP-ACP has been reported to have topical anti-cariogenic effects because of its ability to stabilize calcium and phosphate in an amorphous state [12-14]. Daily use of casein phosphopeptide (CPP)-amorphous calcium phosphate (ACP) has been shown to increase enamel re-mineralization and mineralization of artificial sub-surface enamel lesions increased more than 78% compared to control samples state [15,16]. The proposed anti-cariogenic mechanism of CPP-ACP involves the incorporation of nano-complexes into dental plaque and onto the tooth surface, thereby acting as a calcium and phosphate reservoir [17]. In addition, CPP-ACP can be incorporated into supragingival dental plaque by binding to the surfaces of bacterial cells and to adsorbed macromolecules on tooth surfaces. These interactions can cause the formation of a less-cariogenic plaque [18].

Microabrasion is an effective treatment approach for the cosmetic improvement of long-standing post-orthodontic demineralized enamel lesions [5]. Microabrasion has many applications and has been widely used for the removal of superficial non-carious enamel defects [5]. Gelgör et al. [19] used microabrasion on artificial white lesions and concluded that local enamel decalcifications may be sufficiently eliminated by microabrasion, without any detrimental effect on enamel.

As a new way of treating demineralization, the use of enamel-penetrating, low-viscosity light-curing resins has been suggested to inhibit further demineralization [20]. This method aims to occlude the pores of untreated enamel lesions and, thus, prevent acid penetration into the lesions by forming a diffusion barrier within the enamel lesion [21]. Ekizer et al. [21] reported that resin infiltration of the porous lesion structures might strengthen the lesion mechanically and prevent caries formation.

Controlling dental plaque before and during fixed orthodontic treatment, without compromising shear bond strength (SBS) of brackets, has always been an area of research in orthodontics [22]. Although there are many studies concerning the effect of different demineralization treatment methods on SBS, there is not a total evaluation of the methods reported in the literature, under the same conditions. Therefore, the aim of this study was to evaluate and compare the effects of various demineralization treatment methods on the SBS of orthodontic brackets bonded to demineralized human premolar teeth. The research hypotheses were as follows:

(1) Different demineralization treatment methods have no effect on the SBS;
(2) Different demineralization treatment methods differ from each other in terms of SBS; and
(3) There is no statistically significant difference in fracture mode of brackets after the application of different demineralization treatment methods.

Materials and methods

A power analysis established by G*Power Version 3.1.3 (Franz Faul, Universität Kiel, Germany) software, based on an equal ratio among groups, and a sample size of 140 teeth would give more than 87% (actual power = 0.877,712,7) power to detect significant differences with 0.35 effect size and at the $\alpha = 0.05$ significance level (critical $F = 2.167,423,2$; non-centrality parameter $\lambda = 17.150,000,0$).

Specimen preparation

A total of 140 premolars extracted before orthodontic treatment were included in the present study. The teeth were stored at room temperature in distilled water containing 0.2% thymol to inhibit bacterial growth until use (for a maximum of 1 month). The teeth were cleaned and polished with a fluoride-free pumice slurry and rubber cups for 10 s and thoroughly washed and dried by exposure to oil-free air stream. In trans-illumination examination, the teeth showed healthy enamel on the buccal surface, without attrition, fracture, restoration, congenital anomalies and structural defects. Teeth with caries, hypoplastic areas, restorations and surface abnormalities were excluded from the study.

The teeth were mounted vertically in a cold-curing acrylic (Orthocryl; Dentaurum, Ispringen, Germany) cylinder and the long axis of each tooth was aligned vertically to the base of the cylinder. The teeth were randomly divided into seven groups containing 20 teeth per group considering the demineralization treatment methods to be used, by using a random numbers table.

Demineralization procedure

The enamel surfaces were exposed to demineralizing solution considering the technique first described by Reynolds [13], at 37°C for 3 weeks at pH 4.8. The composition of the solution was 40 mL of 0.1 mol/L lactic acid, 500 mg/L hydroxyapatite and 20 g/L Carbopol C907.

Pre-treatment methods

Pre-treatment methods used were as follows (Table I):

- *Group I* (Control group): No enamel demineralization procedure was performed.
- *Group II*: Brackets were directly bonded to the demineralized enamel surface without any pre-treatment methods.
- *Group III*: CPP-ACP paste (GC Tooth Mousse, Asia Pty. Itabashi- Ku, Tokyo, Ltd, Japan) was applied to the demineralized enamel surface before bonding. The paste was applied onto the enamel

Table I. Pre-treatment methods used in the study.

Groups	Demineralization treatment	Manufacturer	Composition
Group I	Control group (no demineralization)		
Group II	No pre-treatment		
Group III	CPP-ACP	GC Tooth Mousse; GC International, Itabashi- Ku, Tokyo, Japan	Pure water, glycerol, Casein Phosphopeptide-Amorphous Calcium Phosphate, d-sorbitol, xylitol, CMC-Na, propylene glycol, H_2O, SiO_2, TiO_2, ZnO_2, H_3PO_4, MgO_2, guar gum, sodium saccharin,ethyl *p*-hydroxybenzoate, butyl *p*-hydroxybenzoate and propyl *p*-hydroxybenzoate.
Group IV	Fluoride varnish	Bifluorid 12; Voco-GmbH, Cuxhaven, Germany	Sodium fluoride, calcium fluoride. ethyl acetate, pyroxylin, colloidal silicon dioxide, clove.
Group V	Microabrasion-1	Hand made	8% hydrochloric acid, fine-powdered pumice, glycerine.
Group VI	Microabrasion-2	Opalustre, UltraDent, UT	6.6% hydrochloric acid, silicon carbide.
Group VII	Resin infiltration	Icon®; DMG, Hamburg, Germany	Icon-Etch: hydrochloric acid, pyrogenic silicic acid, surface-active substances; Icon-dry: 99% ethanol; Icon-Infiltrant: methacrylate-based resin matrix, initiators, additives.

for 5 min and then rinsed with de-ionized water. It was re-applied after 6 h and this procedure was repeated 10-times. Between each application, all teeth were kept 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 [23].

- *Group IV*: Fluoride varnish (Bifluorid 12; Voco-GmbH, Cuxhaven, Germany) was applied to the demineralized enamel surface before bonding according to the manufacturer's recommendations. The varnish was applied in a thin layer and then allowed to set.
- *Group V*: Microabrasion therapy [24] consisting of a handmade mixture prepared with 18% hydrochloric acid and fine pumice powder was applied to the demineralized enamel surface using an electronic toothbrush Braun Oral-B Plaque Control 3D, Braun, Kronberg, Germany) before bonding. The mixture was applied for 3 min and then rinsed off with de-ionized water. This therapy was re-applied after 6 h and repeated 5-times. Between each application, all teeth were kept in artificial saliva, of which the composition was defined above.
- *Group VI*: A microabrasion agent (Opalustre, UltraDent, South Jordan, UT, USA) was applied to the demineralized enamel surface before bonding. The agent was applied according to the same protocol as described for Group V.
- *Group VII*: A resin infiltrant (Icon®; DMG, Hamburg, Germany) was applied to the demineralized enamel surface before bonding according to the manufacturer's recommendations. Icon-Etch was applied for 120 s, rinsed with water for 30 s and air-dried. Icon-Dry was then applied for 30 s and air-dried. Finally, Icon-Infiltrant was applied for 180 s, light-cured for 40 s, re-applied for 60 s and light-cured for 40 s.

Bonding procedure

The stainless steel premolar brackets Equilibrium® (Dentaurum, Ispringen, Germany) without hook were bonded onto the middle part of the anatomic crowns of buccal surfaces in all groups. Before bonding of brackets, the teeth were etched with 37% phosphoric acid (Gel Etch, 3M Unitek, Monrovia, CA), rinsed with water and air-dried with an oil-free source for 15 s each. Transbond XT primer (3M Unitek) was applied as a thin, uniform coat to the etched enamel surfaces and cured for 10 s using LED. Transbond XT (3M Unitek) adhesive resin was placed onto bracket surfaces. The bracket was placed onto the tooth surface, adjusted to its final position and pressed firmly into place. Excessive sealant and adhesive were removed from the periphery of the bracket base to keep each bonding area uniform. The exposure was performed from both the mesial and distal sides for 20 s, respectively. The emitting tip of the LED was held at 45° to the tooth surface and as close to the bracket as possible.

Test procedure

After bracket bonding, all specimens were stored in distilled water at 37°C for 24 h and later subjected to thermocycling 10,000-times in distilled water between 5°–55°C, with a dwell time in each bath of 30 s and a transfer time of 15 s.

The SBS test was performed using a chisel edge, mounted on the crosshead of a universal testing machine (Elista TSTM 02500, Elista Corp, Istanbul, Turkey). The specimens were subjected to stress at the bracket and enamel interface from a vertical direction, with a crosshead speed of 0.5 mm/min. The maximum shear force necessary to debond each bracket was recorded in Newtons and then converted into megapascals by dividing the value by the surface area of the bracket base. The average base surface areas of the brackets were calculated as 12.2 mm^2 by using a digital caliper (Absolute Digimatic, Mitutoyo Corp, Kawasaki, Japan).

Adhesive remnant index

After debonding, the enamel surface of each tooth was examined by the same operator (M.A.) under an optical microscope (CX41, Olympus, Tokyo, Japan) at 40× magnification to determine the amount of residual adhesive remaining on each tooth. The amount of adhesive left on the enamel surface was scored for each tooth using the modified adhesive remnant index (ARI) [25]. ARI scores were determined as (1) all adhesive remaining on enamel surface; (2) more than 50% adhesive remaining on enamel surface; (3) less than 50% adhesive remaining on enamel surface; and (4) no adhesive remaining on the enamel surface. When a low ARI score with all the composite remaining on the enamel surface is available; the risk of enamel fracture during debonding decreases.

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS for windows, version 17.0 SPSS Inc, Chicago, IL). Normal distribution of the data was verified with the Kolmogorov-Smirnov test. 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 analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) post-hoc test were used for the comparison of SBS values between groups. G- test was used to determine statistically significant differences in the ARI scores among groups. Statistical significance was determined at a probability value of $p < 0.05$.

Results

Descriptive statistics including the mean, standard deviation, minimum and maximum SBS values in MPa are shown in Table II. The statistical analysis revealed statistically significant differences between the SBS values of the groups ($p < 0.001$). SBS values significantly reduced after demineralization as compared with the control group ($p < 0.001$). The group subjected to resin infiltrant (Group VII) produced the highest mean SBS values (19.1 ± 1.4 MPa) and the

Table II. Descriptive statistics and shear bond strength comparisons of seven groups.

Group	n	Mean	SD	Min	Max	Significance ANOVA	Tukey*
Group I	20	18.8	2.0	15.8	22.4	$p < 0.001$; $F = 191.697$	E
Group II	20	6.8	1.1	5.0	9.3		A
Group III	20	16.2	1.4	13.9	18.5		D
Group IV	20	11.5	1.2	9.2	13.8		B
Group V	20	12.6	1.5	9.8	15.8		B
Group VI	20	14.8	1.1	11.6	16.5		C
Group VII	20	19.1	1.4	16.1	21.0		E

Min, Minimum; Max, Maximum; SD, Standard Deviation.
*Groups with different letters are significantly different from each other.
Group 1: Control; Group II: Demineralization, no treatment; Group III: CPP-ACP paste, Group IV: fluoride varnish, Group V: microabrasion with a mixture prepared with 18% hydrochloric acid and fine pumice powder, Group VI: microabrasion with an agent, Group VII: resin infiltrant.

group subjected to demineralization without any treatment method (Group II) yielded the lowest mean SBS values (6.8 ± 1.1 MPa). No significant differences were found between control (Group I) and resin infiltrant group (Group VII) and between the groups subjected to fluoride varnish (Group IV) and handmade microabrasion mixture (Group V). In the comparison of microabrasion therapies, statistically significant differences were found between groups ($p < 0.001$). The microabrasion agent Opalustre had better SBS values than the handmade mixture. Application of resin infiltrant after demineralization led to significantly better adhesion of the brackets as compared with fluoride varnish, CPP-ACP or microabrasion ($p < 0.001$).

The frequency of ARI scores and their comparison are shown in Table III. G-test revealed statistically significant differences between all groups ($p < 0.01$). While no detachment was found at the enamel–composite interface in the resin infiltrant group (Group VII), 80% of the total detachments occurred between the enamel–composite interfaces in the demineralized teeth without treatment (Group II).

Discussion

Enamel demineralization is an undesirable but common complication of orthodontic fixed appliance therapy. This study was performed to evaluate the effects of different treatment methods on the SBS of orthodontic brackets bonded to demineralized enamel surfaces. The specificity of this study with respect to other studies is the total evaluation of frequently used technique and to provide clinicians to keep their knowledge up to date. Based on the

Table III. Fracture modes after shear bond strength testing.

Groups	*n*	1	2	3	4
Group I	20	4 (20%)	7 (35%)	8 (40%)	1 (5%)
Group II	20	0	0	4 (20%)	16 (80%)
Group III	20	0	5 (25%)	12 (60%)	3 (15%)
Group IV	20	0	2 (10%)	8 (40%)	10 (50%)
Group V	20	0	5 (25%)	8 (40%)	7 (35%)
Group VI	20	0	9 (45%)	4 (20%)	7 (35%)
Group VII	20	5 (25%)	6 (30%)	9 (45%)	0

$p < 0.01$; ARI scores, 1: all adhesive remaining on enamel surface; 2: more than 50% adhesive remaining on enamel surface; 3: less than 50% adhesive remaining on enamel surface; 4: no adhesive remaining on the enamel surface.
Group 1: Control; Group II: Demineralization, no treatment; Group III: CPP-ACP paste, Group IV: fluoride varnish, Group V: microabrasion with a mixture prepared with 18% hydrochloric acid and fine pumice powder, Group VI: microabrasion with an agent, Group VII: resin infiltrant.

results, the research hypotheses of this study were rejected.

Thermocycling is a widely used artificial aging methodology [26] and, in the literature, various thermocycling protocols have been used by different authors [27,28]. A literature review [29] concluded that 10,000 cycles corresponds approximately to 1 year of *in-vivo* functioning. Also it was reported that more cycles are needed to mimic long-term bonding effectiveness [26]. In the current study, 10,000 cycles between 5°–55°C with a dwell time of 30 s were used as the thermocycling regimen.

To simulate the moisture and temperature conditions in the oral cavity, all bonded samples were stored in distilled water at 37°C for 24 h before SBS testing [30]. In the literature; various storage media have been used to store teeth like water, saline, artificial saliva, etc. However, it was reported that the storage media does not alter the SBS [31].

Artificially demineralized surfaces were used in the present study as described by Reynolds [30]. As shown in many previous studies [6,32,33], demineralization of the enamel surface decreased the SBS of orthodontic brackets. Uysal et al. [33] attributed low SBS to the poor quality of the enamel surface and a lack of resin tags for the formation of mechanical interlocking.

Since its introduction, the use of Tooth Mousse™ (a CPP-ACP) as a topical coating for teeth has been gaining popularity not only because of its remineralization effect, but also because of its caries-preventing effect in long-term clinical use [34]. The results of the present study showed that CPP-ACP application was better than other treatment methods except resin infiltration. Akin et al. [35] compared the effects of CPP-ACP application and microabrasion on treatment of white spot lesions with a control group and concluded that microabrasion was an effective method for the treatment of white spot lesions and followed CPP-ACP with regards to effectiveness.

The effects of fluoride on the prevention of tooth decay and re-mineralization of decalcified enamel have been elaborately described [7,36-38]. Shahabi et al. [7] reported that application of 2% sodium fluoride (NaF) prior to acid etching of demineralized enamel caused a significant increase in bond strength of orthodontic brackets. The results of the present study revealed that fluoride varnish significantly increased the SBS of brackets when compared to those on demineralized enamel without pre-treatment methods. However, SBS values are still lower than the control group. It has been known that the topical application of fluoride can interfere with the etching effect of phosphoric acid on enamel surfaces, resulting in reduced bond strength of orthodontic brackets [39,40].

Application of resin infiltrant after demineralization led to significantly better adhesion of the brackets as compared with fluoride varnish, CPP-ACP or microabrasion. Also, the mean SBS value of the resin infiltrant group (19.1 ± 1.4 MPa) was greater than that of the control group (18.8 ± 2.0 MPa). However, this difference was not statistically significant. Attin et al. [41] reported that pre-treatment with the resin infiltrant was a beneficial approach to increase the SBS of brackets to demineralized enamel, but also noted that resin infiltrant resulted in significantly lower bond strength of the brackets compared with application of the brackets on sound enamel.

In comparison of microabrasion therapies, statistically significant differences were found between groups. The microabrasion agent Opalustre had better SBS values than the handmade mixture. Enamel microabrasion is generally acknowledged to be a well-accepted technique [5]. However, a criticism of the hydrochloric acid and pumice technique relates to potential tooth–tissue loss. Although Opalustre includes a lower amount of hydrochloric acid, the better SBS values may be attributed to the effect of silicon carbide.

The results of ARI score comparisons in the present study indicated that there were significant differences among all groups tested. The most desired situation is a low ARI score with all the composite remaining on the enamel surface [22]. According to the ARI scores, 80% of demineralized teeth without treatment method had no adhesive remaining on the enamel surface. Also, there was a greater frequency of ARI scores of 3 in the control, CPP-ACP and resin infiltrant groups, indicating that the bond between bracket and resin was stronger than that between the resin and enamel.

Reynolds [30] suggested that minimum bond strength of 6–8 MPa was adequate for most orthodontic needs. These bond strengths are considered to be able to withstand masticatory and orthodontic forces. In the present study, although there was a significant difference among the groups, all SBS values achieved were above this minimum requirement. However, the suggested values are based on tensile strength, whereas SBS values were evaluated in the present study.

In the current study, every effort was made to standardize the testing procedure in an attempt to mimic the clinical situations faithfully. However, it is acknowledged that *in vitro* bond strength testing is not truly representative of the highly demanding intra-oral conditions and at best gives only an indication of possible clinical performance of the material tested. The validity of the results depends on how appropriate and representative the laboratory test conditions are and, although in the present study each step of the protocol was strictly followed, the findings should be interpreted with caution.

Conclusion

Based on the results of the present study, the following conclusions can be drawn:

- Demineralization significantly lowered the SBS values.
- All demineralization treatment methods improve the bonding to the demineralized enamel surface.
- Application of resin infiltrant after demineralization led to significantly better adhesion of the brackets as compared with fluoride varnish, CPP-ACP or microabrasion.
- CPP-ACP is more efficient than both microabrasion methods in demineralization treatment and provides higher SBS values.
- There was significant evidence to suggest a statistical difference between the failure characteristics of the groups. No enamel detachment was observed in the resin infiltrant group.

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

References

[1] Zachrisson BU, Zachrisson S. Caries incidence and orthodontic treatment with fixed appliance. Scand J Dent Res 1971; 79:183–92.

[2] Boersma JG, Vander Veen MH, Lagerweij MD, Bokhout B. Caries prevalence measured with QLF after treatment with fixed orthodontic appliances: influencing factors. Caries Res 2005;39:41–7.

[3] Mitchell L. Decalcification during orthodontic treatment with fixed appliances–an overview. Br J Orthod 1992;19:199–205.

[4] Zachrisson BU. A post-treatment evaluation of direct bonding in orthodontics. Am J Orthod 1977;71:173–89.

[5] Murphy TC, Willmot DR, Rodd HD. Management of post-orthodontic demineralized white lesions with microabrasion: a quantitative assessment. Am J Orthod Dentofacial Orthop 2007;131:27–33.

[6] Shahabi M, Moosavi H, Gholami A, Ahrari F. In vitro effects of several surface preparation methods on shear bond strength of orthodontic brackets to caries-like lesions of enamel. Eur J Paediatr Dent 2012;13:197–202.

[7] Shahabi M, Ahrari F, Mohamadipour H, Moosavi H. Microleakage and shear bond strength of orthodontc brackets bonded to hypomineralized enamel following different surface preparations. J Clin Exp Dent 2014;6:e110–15.

[8] Geiger AM, Gorelick L, Gwinnett AJ, Benson BJ. Reducing white spot lesions in orthodontic populations with fluoride rinsing. Am J Orthod Dentofacial Orthop 1992; 101:403–7.

[9] Sadowsky PL, Retief DH, Bradley EL Jr. Enamel fluoride uptake from orthodontic cements and its effect on demineralization. Am J Orthod 1981;79:523–34.

[10] de Leeuw NH. Resisting the onset of hydroxyapatite dissolution through the incorporation of fluoride. J Phys Chem 2003;108:1809–11.

[11] Benson PE, Shah AA, Millett DT, Dyer F, Parkin N, Vine RS. Fluorides, orthodontics and demineralization: a systematic review. J Orthod 2005;32:102–14.

[12] Rose RK. Effects of an anticariogenic casein phosphopeptide on calcium diffusion in streptococcal model dental plaques. Arch Oral Biol 2000;45:569–75.

[13] Reynolds EC. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. J Dent Res 1997;76:1587–95.

[14] Shen P, Cai F, Nowicki A, Vincent J, Reynolds EC. Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. J Dent Res 2001;80:2066–70.

[15] Reynolds EC, Cai F, Shen P, Walker GD. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. J Dent Res 2003;82:206–11.

[16] Cai F, Shen P, Morgan MV, Reynolds EC. Remineralization of enamel subsurface lesions in situ by sugar-free lozenges containing casein phosphopeptide-amorphous calcium phosphate. Aust Dent J 2003;48:240–3.

[17] Sudjalim TR, Woods MG, Manton DJ, Reynolds EC. Prevention of demineralization around orthodontic brackets in vitro. Am J Orthod Dentofacial Orthop 2007;131:705.e1–9.

[18] Rose RK. Binding characteristics of streptococcus mutans for calcium and casein phosphopeptide. Caries Res 2000;34: 427–31.

[19] Gelgör I, Ercan E, Akyalcin S, Dülgergil T, Catalbas B. A scanning electron microscope investigation into white spot lesion removal with microabrasion approach. Turkiye Klinikleri J Dental Sci 2009;15:11–17.

[20] Paris S, Hopfenmuller W, Meyer-Lueckel H. Resin infiltration of caries lesions: an efficacy randomized trial. J Dent Res 2010;89:823–6.

[21] Ekizer A, Zorba YO, Uysal T, Ayrikcil S. Effects of demineralization-inhibition procedures on the bond strength of brackets bonded to demineralized enamel surface. Korean J Orthod 2012;42:17–22.

[22] Tabrizi A, Cakirer B. A comparative evaluation of casein phosphopeptide-amorphous calcium phosphate and fluoride on the shear bond strength of orthodontic brackets. Eur J Orthod 2011;33:282–7.

[23] Donmez N, Belli S, Pashley DH, Tay FR. Ultrastructural correlates of in vivo/in vitro bond degradation in self-etch adhesives. J Dent Res 2005;84:355–9.

[24] Gelgor IE, Buyukyilmaz T. A practical approach to white spot lesion removal. World J Orthod 2003;4:152–6.

[25] Montasser MA, Drummond JL. Reliability of the Adhesive Remnant Index score system with different magnifications. Angle Orthod 2009;79:773–6.
[26] De Munck J, Van Landuyt K, Coutinho E, Poitevin A, Peumans M, Lambrechts P, et al. Micro-tensile bond strength of adhesives bonded to Class-I cavity-bottom dentin after thermo-cycling. Dent Mater 2005;21:999–1007.
[27] Cehreli ZC, Kecik D, Kocadereli I. Effect of self-etching primer and adhesive formulations on the shear bond strength of orthodontic brackets. Am J Orthod Dentofacial Orthop 2005;127:573–9.
[28] Turgut MD, Attar N, Korkmaz Y, Gokcelik A. Comparison of shear bond strengths of orthodontic brackets bonded with flowable composites. Dent Mater J 2011;30:66–71.
[29] Gale MS, Darvell BW. Thermal cycling procedures for laboratory testing of dental restorations. J Dent 1999;27:89–99.
[30] Reynolds IR. A review of direct orthodontic bonding. Br J Orthod 1975;2:171–8.
[31] Oesterle LJ, Shellhart WC. Effect of aging on the shear bond strength of orthodontic brackets. Am J Orthod Dentofacial Orthop 2008;133:716–20.
[32] Baysal A, Uysal T. Do enamel microabrasion and casein phosphopeptide-amorphous calcium phosphate affect shear bond strength of orthodontic brackets bonded to a demineralized enamel surface? Angle Orthod 2012;82:36–41.
[33] Uysal T, Baysal A, Uysal B, Aydinbelge M, Al-Qunaian T. Do fluoride and casein phosphopeptide-amorphous calcium phosphate affect shear bond strength of orthodontic brackets bonded to a demineralized enamel surface? Angle Orthod 2011;81:490–5.
[34] Yengopal V, Mickenautsch S. Caries preventive effect of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP): a meta-analysis. Acta Odontol Scand 2009;67:321–32.
[35] Akin M, Basciftci FA. Can white spot lesions be treated effectively? Angle Orthod 2012;82:770–5.
[36] Bibby BG. Preliminary report on the use of sodium fluoride applications in caries prophylaxis. J Dent Res 1942;21:314.
[37] Hicks M, Silverstone L. The effect of acid-etching on caries-like lesions treated with stannous fluoride. J Dental Res 1983;62:783–8.
[38] Hicks MJ, Silverstone LM, Flaitz CM. A scanning electron microscopic and polarized light microscopic study of acid-etching of caries-like lesions in human tooth enamel treated with sodium fluoride in vitro. Arch Oral Biol 1984;29:765–72.
[39] Garcia-Godoy F, Hubbard GW, Storey AT. Effect of fluoridated etching gel on enamel morphology and shear bond strength of orthodontic brackets. Am J Orthod Dentofacial Orthop 1991;100:163–70.
[40] Cacciafesta V, Sfondrini MF, Calvi D, Scribante A. Effect of fluoride application on shear bond strength of brackets bonded with a resin-modified glass-ionomer. Am J Orthod Dentofacial Orthop 2005;127:580–3.
[41] Attin R, Stawarczyk B, Keçik D, Knösel M, Wiechmann D, Attin T. Shear bond strength of brackets to demineralize enamel after different pretreatment methods. Angle Orthod 2012;82:56–61.