Acta Odontologica Scandinavica. 2014; 72: 825–830

informa
healthcare

ORIGINAL ARTICLE

Stability of two resin combinations used as sealants against toothbrush abrasion and acid challenge *in vitro*

ENVER YETKINER[1], FLORIAN JUST WEGEHAUPT[2], RENGIN ATTIN[3], ANNETTE WIEGAND[4] & THOMAS ATTIN[2]

[1]*Department of Orthodontics, University of Ege*, Izmir, Turkey, [2]*Clinic for Preventive Dentistry, Periodontology and Cariology*, [3]*Clinic for Orthodontics and Pediatric Dentistry, University of Zürich, Switzerland, and* [4]*Department of Preventive Dentistry, Periodontology and Cariology, University of Göttingen, Germany*

Abstract
Objective. To test the stability of two conventional adhesives when combined with a low-viscosity caries infiltrant used for sealing sound enamel against toothbrush abrasion and acid challenge *in vitro*. ***Materials and methods.*** Bovine enamel discs (Ø = 3 mm) randomly assigned to three groups (*n* = 10/group) were etched with 37% phosphoric acid for 30 s and treated with resins of different monomer contents forming three test groups: (1) Untreated specimens (Control); (2) Infiltrant (Icon, DMG) + conventional enamel bonding adhesive (Heliobond, Ivoclar Vivadent); and (3) Infiltrant + conventional orthodontic adhesive (Transbond XT Primer, 3M Unitek). All specimens were immersed in hydrochloric acid (pH 2.6) for up to 9 days, during which they were exposed to 1825 toothbrush-strokes per day. Calcium dissolution was assessed using Arsenazo III method at 24-h intervals. Data were analyzed by Kruskal-Wallis and Wilcoxon signed ranks tests. ***Results.*** Cumulative calcium dissolution for the untreated specimens (39.75 ± 7.32 μmol/ml) exceeded the sealed groups (Icon + Heliobond: 23.44 ± 7.03 μmol/ml; Icon + Transbond XT Primer: 22.17 ± 5.34 μmol/ml). Untreated specimens presented a relatively constant calcium dissolution rate throughout the experimental period, whereas the sealed groups presented a gradual increase indicating weakening of the seal by toothbrush abrasion. Both sealed groups presented significantly lower daily calcium dissolution at all time points compared to the control, except for Group 2 on the last measurement day. ***Conclusions.*** Low-viscosity caries infiltrant application on sound enamel prior to conventional resin application provided a protective effect against enamel demineralization, but this effect was not stable when challenged mechanically by toothbrush abrasion.

Key Words: *caries infiltrant, sealant, white spot lesion*

Introduction

Maintaining oral hygiene during orthodontic treatment with bonded appliances is a challenging task. Retentive areas within the design of attachments lead to plaque accumulation that harbors cariogenic bacteria. Daily-consumed carbohydrates get fermented by these bacteria, which leads to the formation of sub-surface enamel demineralizations, so called white spot lesions (WSLs) [1]. Prevalence of these acquired surface lesions is relatively high, affecting more than 50% of orthodontic patients [2,3]. WSLs can appear rapidly, as fast as 3–4 weeks, which happens to be the subsequent appointment following bracket placement [4]. Therefore, protection of healthy mineralized hard tissues against demineralization during orthodontic treatment is a critical problem [1].

Efficacy of preventive measures for this negative side-effect has been questioned previously [1,5,6]. Prevention methods mainly aim at enamel remineralization and cariogenic bacteria inhibition [1,5,6]. Remineralization of WSLs could be achieved to some extent using topical fluoride applications and casein phosphopeptide-amorphous calcium-phosphate (CPP-ACP) containing pastes. Topical fluoride forms more stable compounds against demineralization such as Fluor-hydroxyapatite mixed crystals and calcium fluoride precipitates, whereas CPP-ACP increases the calcium and phosphate content in the saliva favoring remineralization [7–9]. However,

Correspondence: Enver Yetkiner, University of Ege, Department of Orthodontics, Izmir, Turkey. Tel: +90 232 388 0326. Fax: +90 232 388 0325.
E-mail: eyetkiner@hotmail.com

(Received 7 January 2014; accepted 11 March 2014)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.3109/00016357.2014.913197

patient compliance, which is a major factor determining the success of these methods, decreases throughout the treatment period [10]. Thus, preventive measures that do not require compliance were considered to be more predictable in such high-risk patients [1,5,6]. Clinically it has been shown that once WSLs are formed, they do not completely disappear by means of remineralization treatment unless removed mechanically by micro-abrasion, infiltrated with a low-viscosity resin or treated in a restorative fashion [5,11,12]. These treatment methods involve removal of healthy and affected dental tissues to some extent. Therefore, preventive measures independent of patient compliance, such as sealants which cover the susceptible enamel prior to bracket placement in order to form a caries-protective layer, have been a focus of interest in recent years [13–16].

In principle, sealants cover the buccal surface adjacent to brackets, providing protection as a physical barrier during treatment [14]. However, this shielding layer is subjected to challenges such as acid attacks from bacterial plaque and sugary soft drinks as well as daily toothbrushing and food consumption, which might impair the seal [13,14]. Recently, a low viscosity caries infiltrant with high penetration ability has been reported to present a protective effect against demineralization on sound and demineralized enamel [15,16]. This new resin has originally been developed to arrest WSL progression and to prevent further demineralization by forming a resistant condensed layer via infiltrating the enamel [11,12]. In contrast to conventional adhesives, where the resin remains on the surface as a covering coat, this infiltrant presents capillary penetration into the pores of etched enamel creating a diffusion barrier within the mineralized structure due to its low-viscosity and superior surface wetting abilities [12,17,18]. In addition, new retention areas for plaque accumulation at the infiltrated margins were claimed to be avoided [12,15]. The protective effect of this infiltrant against demineralization when applied alone was shown to be inferior to conventional resins [15]. On the other hand, its improving effect on protection when combined with conventional adhesives and the integrity of these surfaces in terms of bonding performance has been shown previously [16,18]. However, the stability of such resin combinations in terms of resistance against mechanical challenges simulating real life settings has not been questioned.

Therefore, the objective of this study was to evaluate the toothbrush abrasion and acid attack resistance of two conventional adhesive resins with varying monomer blends applied subsequently after the infiltration of sound enamel with a low-viscosity caries infiltrant *in vitro*. The null hypothesis tested was that the protective effect provided by the combinations of resins would not maintain its effect against toothbrush abrasion, representing a 2-year period *in vivo*.

Materials and methods

Bovine enamel, two conventional adhesives (Heliobond, Ivoclar Vivadent, Schaan, Liechtenstein; Transbond XT Primer, 3M Unitek, Monrovia, CA, USA) and low-viscosity caries infiltrant (Icon, DMG, Hamburg, Germany) were employed in this *in vitro* study. The adhesives contained varying ratios of Bis-phenol-A-glycidylmethacrylate (Bis-GMA) and Triethyleneglycol dimethacrylate (TEGDMA). The low-viscosity caries infiltrant was pure TEGDMA based. Chemical compositions of the materials are summarized in Table I.

Specimen preparation

Crowns of bovine mandibular incisors ($n = 10$) stored in 0.5% chloramine solution at 4°C no longer than 6 months were initially cut from their roots. Three enamel discs with 3 mm diameter were cut from the labial aspect of each tooth using a custom-made diamond-coated trephine (80 μm, Intensiv SA, Lugano-Grancia, Switzerland). Discs were flattened from the bottom to ~2 mm in height (Struers, Birmensdorf, Switzerland). Each disc was assigned to three groups assuring equal distribution of incisal,

Table I. Chemical compositions of the materials according to manufacturers' data sheets.

Product	Chemical composition	Manufacturer
Icon (Infiltrant)	TEGDMA based resin matrix, Initiators - additives	DMG, Hamburg, Germany Batch no. 634902
Transbond XT Primer	bis-GMA 45–55% TEGDMA 45–55% 4-dimethylaminobenzene ethanol <0.5% camphorquinone <0.3% hydroquinone <0.03%	3M Unitek, Monrovia, CA, USA Batch no. 290791
Heliobond	bis-GMA 50–100% TEGDMA 25–50% Initiators - stabilizers	IvoclarVivadent, Schaan, Liechtenstein Batch no. 30513

bis-GMA, Bis-phenol-A-glycidylmethacrylate; TEGDMA, Triethyleneglycol dimethacrylate.

medial and gingival sections per group. They were embedded with their labial surfaces exposed for sealing procedures in auto-polymerizing resin (Paladur, Heraeus Kulzer, Wehrheim, Germany) in cylindrical molds (6 mm diameter, 3 mm thickness). Embedded specimens were ground flat and polished with water-cooled carborundum discs (1200, 2400 and 4000 grit, waterproof silicon carbide paper, Struers, Erkrath, Germany). Specimens were stored in distilled water (grade 3) until sealing procedures.

Sealing procedure

All specimens were etched with 37% H_3PO_4 (Orbis Dental, Münster, Germany) for 30 s and rinsed with water for 30 s. After drying, specimens were treated as follows:

(1) *Control*: Untreated specimens, serving as the control group.
(2) *Infiltrant + conventional enamel bonding adhesive*: Ethanol (Icon-Dry) was applied for 30 s and air-dried for 10 s. Low-viscosity caries infiltrant (Icon) was applied in one coat with a micro-brush covering the whole enamel surface, left to set for 180 s, light-cured for 60 s; a second layer was applied, left to set for 60 s and light-cured for 40 s. Conventional enamel bonding adhesive (Heliobond) was applied in one coat with a micro-brush covering the whole enamel surface and light-cured for 60 s.
(3) *Infiltrant + conventional orthodontic adhesive*: Icon was applied as described under point 2. Then, the conventional orthodontic adhesive (Transbond XT Primer) was applied in one coat with a micro-brush and light-cured for 60 s.
(4) All specimens were stored in distilled water for 24 h at 37°C.

Toothbrush abrasion and acid challenge cycle

Each specimen was immersed in 8 ml hydrochloric acid (HCl, 2500 μmolH$^+$/l, pH 2.6) under constant motion for the first day and Ca-dissolution measurement was performed at the end of 24 h. Immediately after, toothbrush abrasion was done and the specimens were placed in HCl starting the acid challenge-toothbrush abrasion cycles. Ca-dissolution measurements and toothbrush abrasion cycles were repeated for 8 consecutive days every 24 h.

Toothbrush abrasion was performed using a two-axis brushing machine (Willytec GmbH, Feldkirchen-Westerham, Germany) with 2 N force [19]. A toothbrush with medium bristle stiffness (Paro M43, Esro, Kilchberg, Switzerland) was used. Daily, 1825 brushing strokes (BS, 30 strokes per minute) were administered representing 3 months *in vivo* conditions [19]. The toothpaste slurry was prepared with fluoridated dentifrice (Elmex, GABA, Therwil, Switzerland, RDA 77) and distilled water in 1:2 (w/w) proportion. Two milliliters of slurry for each brushing session was used, assuring that the specimens were sufficiently covered. After each tooth-brushing session, specimens were rinsed thoroughly under running water for removing toothpaste remnants and finally rinsed with distilled water prior to re-immersion in HCl.

Assessment of sealant stability

Sealing effect of the surface treatments was quantified by the amount of Ca released from the specimens into the acid solution. Calcium concentrations were colorimetrically assessed in a flat micro-plate reader (Molecular Devices, Ismaning/Munich, Germany) using Arsenazo III method as previously described by Attin et al. [20]. Arsenazo III reacts with calcium to form a bluish-purple complex. Intensity of the color developed is proportional to the calcium concentration and can be determined photometrically according to Beer-Lambert law. For each measurement, 10 μl of the acid solution were added to the wells of a micro-titre plate and were mixed with 100 μl of the color reagent (Fluitest CA AIII R1, Biocon Diagnostik, Vöhl/Marienhagen, Germany). Absorbance was read at 650 nm. Measurements were performed at 25°C.

Statistical analysis

A sample size of 10 in each group was calculated to have 80% power to detect a difference in means of 2.2 μmolCa/ml assuming that the common standard deviation is 1.4 using a two group *t*-test with a 0.017 two-sided significance level of Bonferroni correction. The assumption of approximate normal distribution was investigated by Kolmogorov–Smirnov and Shapiro–Wilk tests. As the data were not normally distributed, Kruskal-Wallis test was applied to analyze possible differences between the groups and at each time point.

To evaluate the stability of different sealing measures, difference in Ca dissolution of day 1 and day 9 was compared with 0 level using Wilcoxon signed ranks test. Significant differences were interpreted as a loss of sealing effect. This was followed by Kruskal-Wallis tests, separately for all combinations of two group comparisons. Level for significance was set at $p < 0.05$.

Results

Cumulative calcium dissolution of the control specimens over 9 days was higher than the sealed groups (Figure 1). Icon + Transbond XT Primer presented lower Ca dissolution at all measurement time points with an increasing Ca dissolution tendency. Icon + Heliobond also provided lower Ca loss

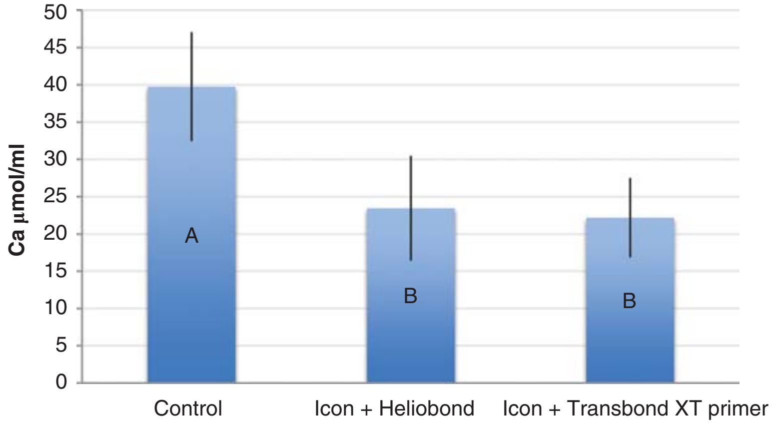

Figure 1. Cumulative calcium dissolution in μmol/ml after 9 days. Groups that are not significantly different are marked with same capital letters.

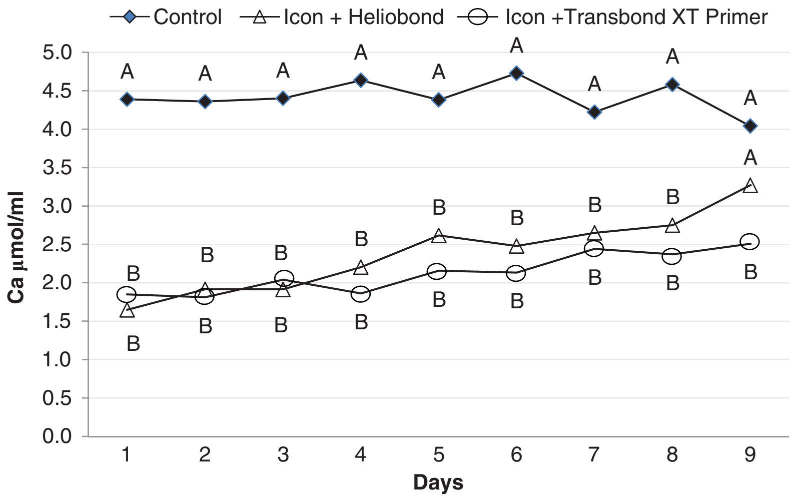

Figure 2. Mean Ca dissolution of groups per day. Groups that are not significantly different are marked with same capital letters (read vertically).

throughout the experimental period except the ninth day with significantly higher Ca dissolution rate than Icon + Transbond XT Primer. The daily Ca dissolution and comparisons between groups are summarized in Figure 2. Comparison of Ca dissolution of day 1 and day 9 with 0 level indicated that both resin combinations could not maintain stability of the protective layer (Figure 3).

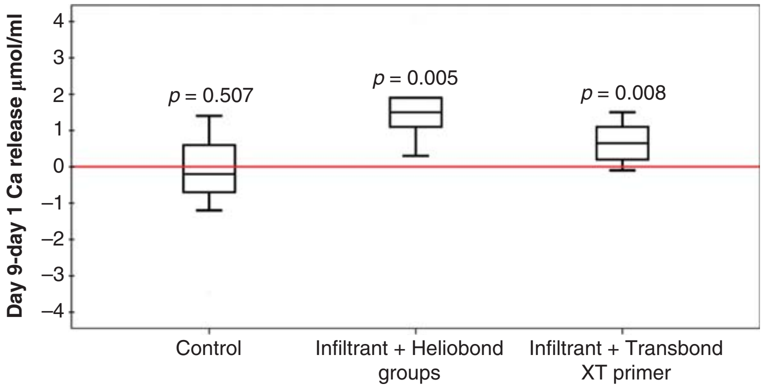

Figure 3. Difference in Ca dissolution of day 1 and day 9 compared with level 0 (horizontal line). Significant difference from this level is interpreted as a loss of protective effect.

Discussion

In this *in vitro* study, stability of two conventional adhesives combined with a low-viscosity caries infiltrant (Icon + Heliobond and Icon + Transbond XT Primer) was evaluated in terms of prevention against demineralization on sound enamel after toothbrush abrasion. The protective effect presented a declining tendency for both groups during the mechanical challenge caused by the toothbrushing abrasion. Although the Ca dissolution was significantly lower than the untreated controls, both groups could not maintain stability of the sealing barrier, thus the null hypothesis cannot be rejected.

Etched enamel adjacent to orthodontic brackets are usually covered with the adhesive that is used to wet the surface for better penetration of resin tags for mechanical interlocking [4,13,14]. This thin layer seals the mineralized tissue underneath against acid challenges resulting from cariogenic bacteria and soft drinks as well as being challenged mechanically itself by toothbrush abrasion and food consumption [13,14]. In this present study, these conditions were simulated by immersion of the specimens in HCl and application of toothbrush abrasion. The pH of the HCl acid used was significantly lower than that of the organic acids produced by bacteria in order to increase the quantity of Ca dissolved for generating detectable amounts in short time periods [20]. In addition, it was assumed that the resin combinations performing well under these highly demineralizing conditions would also be able to show the same relative protective effect against demineralization caused by weaker acids. Toothbrush abrasion was applied following the assumption of Wiegand and Attin [19] and the settings were adjusted to 2 N force, 10 strokes per specimen and two brushing cycles per day. Concurrently, 1825 strokes representing 3 months *in vivo* were applied each day and the specimens were subjected to acid challenge under constant motion for another 24 h cycle following brushing. Average orthodontic treatment duration of 2 years *in vivo* was mimicked at the end of 8 days in terms of brushing. Yet, two possible limitations of the present study regarding these two test conditions are absence of remineralization and intense application of toothbrush abrasion. In real-life settings, remineralization provided by saliva and fluoridated toothpaste would play a role on the mineral content of the sealed surface. Similarly, toothbrushing would not be extended as performed, which would reduce the amount intensity of the mechanical challenge. Despite the fact that these circumstances might have had an influence on the quantity of calcium dissolution during testing, they should not change the findings fundamentally.

Test materials of Icon + Heliobond and Icon + Transbond XT Primer were chosen regarding their varying Bis-GMA/TEGDMA ratio and their previously reported protective effect against demineralization implemented in a previous study [16]. In principle, penetration efficacy of such resins into porous substrates is determined by their viscosity, surface tension and contact angle [12,21,22]. Lower viscosity and surface contact angle result in deeper penetration with higher density [12,21,22]. Accordingly, Icon was shown to lead to a complete, but partially inhomogeneous penetration, whereas Heliobond was shown to induce the formation of a homogenous surface layer, penetrating only the outer surface of the substrate [15]. Therefore, better sealing of the smooth enamel surface was anticipated by combining these resins. In the present study, cumulative Ca dissolution of the sealed groups at the end of the experiment, which represented a 2-year period *in vivo*, was lower than the untreated controls. This finding indicated the sealing effect provided by these resins as reported previously [16]. On the other hand, daily Ca dissolution presented an increasing trend in both sealed groups during the experimental period. When the change in the amount of Ca released between baseline and the end of the experimental period relative to zero level was compared, both groups could not maintain the protective effect, therefore were not stable. These findings can be interpreted as the weakening of the caries infiltrant-conventional enamel adhesive protective layer due to toothbrush abrasion. One possible explanation of this mechanical side-effect might be the absence of filler particles within the formulation of these resins, which was reported to be an influencing factor of the wear resistance of such materials [23]. Interestingly, there was a detectable amount of Ca-dissolution on day 1 prior to any mechanical challenge regarding the sealed groups. Theoretically, there should be no Ca present in the HCl following the first 24 h in the sealed groups since the whole enamel surface is covered with the adhesives. This could be attributed to the Ca blended in the adhesives themselves during the sealing procedure. However, this was not verified as a part of the experimental set-up.

Icon was designed primarily to infiltrate WSLs that manifest porous sub-surface caries formations with a highly mineralized acid resistant outer layer. Conventional 37% H_3PO_4 etching is not capable of altering this resistant surface for reliable orthodontic bonding or resin infiltration [12]. Therefore, 15% HCl acid application has been advocated in order to establish optimal penetration for this material [12,22]. However, this is not the case in sound enamel where H_3PO_4 is capable of creating a micro-retentive surface for successful capillary diffusion. In this present study, H_3PO_4 etching was preferred which was shown previously to be sufficient for Icon to penetrate sound enamel [22].

Penetration time is another important factor determining the rate of resin impregnation and plugging of the gaps formed by etching [24]. Icon was applied on the etched enamel surface with a prolonged penetration time, as described previously, which may be suggested as another factor increasing the chemical and mechanical properties of the combined resins when applied as a sealant layer [24]. However, 180 s application time might not be easy to obtain under clinical conditions, especially on the buccal surfaces of posterior teeth when the patient is in a supine position during bracket placement. Nevertheless, allowing the resin to penetrate as long as possible prior to photo-polymerization should be acknowledged as an improving factor for surface sealing [24].

Alternative materials for sealing the enamel to create a protective layer against demineralization needs initial clinical screening before it becomes an adjunct to conventional bonding procedures in orthodontics. According to the results of the present study, it could be suggested that the application of the caries infiltrant following 37% phosphoric acid etching on sound enamel prior to bracket placement could be an alternative to be used as an additional preventive measure against WSL formation. However, this layer may not persist its protective effect enduring toothbrushing and acid challenge for a representative 2-year orthodontic treatment period *in vivo*.

Acknowledgment

This study was supported by DMG, Hamburg, Germany.

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] Derks A, Katsaros C, Frencken JE, vant't Hof MA, Kuijpers-Jagtman AM. Caries-inhibiting effect of preventive measures during orthodontic treatment with fixed appliances a systematic review. Caries Res 2004;38:413–20.

[2] Tüfekçi E, Dixon JS, Gunsolley JC, Lindauer SJ. Prevalence of white spot lesions during orthodontic treatment with fixed appliances. Angle Orthod 2011;81:206–10.

[3] Hadler-Olsen S, Sandvik K, El-Agroudi MA, Øgaard B. The incidence of caries and white spot lesions in orthodontically treated adolescents with a comprehensive caries prophylactic regimen-a prospective study. Eur J Orthod 2012; 34:633–9.

[4] Gorton J, Featherstone JD. In vivo inhibition of demineralization around orthodontic brackets. Am J Orthod Dentofacial Orthop 2003;123:10–14.

[5] Bergstrand F, Twetman S. A review on prevention and treatment of post-orthodontic white spot lesions-evidence-based methods and emerging technologies. Open Dent J 2011;5:158–62.

[6] Kerbusch AEG, Kuijpers-Jagtman AM, Mulder J, van der Sanden WJM. Methods used for prevention of white spot lesion development during orthodontic treatment with fixed appliances. Acta Odontol Scand 2012;70:564–8.

[7] Zantner C, Martus P, Kielbassa AM. Clinical monitoring of the effect of fluorides on long existing white spot lesions. Acta Odontol Scand 2006;64:115–22.

[8] Beerens MW, van der Veen MH, van Beek H, ten Cate JM. Effects of casein phosphopeptide amorphous calcium fluoride phosphate paste on white spot lesions and dental plaque after orthodontic treatment: a 3-month follow-up. Eur J Oral Sci 2010;118:610–17.

[9] ten Cate JM. Contemporary perspective on the use of fluoride products in caries prevention. Br Dent J 2013;214:161–7.

[10] Cantekin K, Celikoglu M, Karadas M, Yildirim H, Erdem A. Effects of orthodontic treatment with fixed appliances on oral health status: a comprehensive study. J Dent Sci 2011; 6:235–8.

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

[12] Paris S, Meyer-Lueckel H. Inhibition of caries progression by resin infiltration in situ. Caries Res 2010;44:47–54.

[13] Paschos E, Kleinschrodt T, Luedemann-Clementino T, Huth KC, Hickel R, Kunzelmann KH, et al. Effect of different bonding agents on prevention of enamel demineralization around orthodontic brackets. Am J Orthod Dentofacial Orthop 2009;135:603–12.

[14] Tanna N, Kao E, Gladwinn M, Ngan PW. Effects of sealant and self-etching primer on enamel decalcification. Part 1: an in-vitro study. Am J Orthod Dentofacial Orthop 2009; 135:199–205.

[15] Schmidlin PR, Sener B, Attin T, Wiegand A. Protection of sound enamel and artificial enamel lesions against demineralization: caries infiltrant versus adhesive. J Dent 2012;40: 851–6.

[16] Yetkiner E, Wegehaupt FJ, Attin R, Attin T. Caries infiltrant combined with conventional adhesives for sealing sound enamel in vitro. Angle Orthod 2013;83:858–63.

[17] Belli R, Rahiotis C, Schubert EW, Baratieri LN, Petschelt A, Lohbauer U. Wear and morphology of infiltrated white spot lesions. J Dent 2011;39:376–85.

[18] Yetkiner E, Özcan M, Wegehaupt FJ, Wiegand A, Eden E, Attin T. Effect of a low-viscosity adhesive resin on the adhesion of metal brackets on hydrochloric or phosphoric acid etched enamel combined with conventional adhesives. J Adhes Dent 2013;15:575–81.

[19] Wiegand A, Attin T. Design of erosion/abrasion studies–insights and rational concepts. Caries Res 2011;45:53–9.

[20] Attin T, Becker K, Hannig C, Buchalla W, Hilgers R. Method to detect minimal amounts of calcium dissolved in acidic solutions. Caries Res 2005;39:432–6.

[21] Paris S, Meyer-Lueckel H, Colfen H, Kielbassa AM. Penetration coefficients of commercially available and experimental composites intended to infiltrate enamel carious lesions. Dent Mater 2007;23:742–8.

[22] Paris S, Meyer-Lueckel H, Colfen H, Kielbassa AM. Resin infiltration of artificial enamel caries lesions with experimental light curing resins. Dent Mater J 2007;26:582–8.

[23] Shinishin SF, Ghobashy SA, El-Bialy TH. Efficacy of light-activated sealant on enamel demineralization in orthodontic patients: an atomic force microscope evaluation. Open Dent J 2011;5:179–86.

[24] Meyer-Lueckel H, Chatzidakis A, Naumann M, Dörfer CE, Paris S. Influence of application time on penetration of an infiltrant into natural enamel caries. J Dent 2011;39: 465–9.