In vivo effect of antibacterial and fluoride-releasing adhesives on enamel demineralization around brackets: A micro-CT study

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ABSTRACT

Objectives: The aim of this in vivo study was to investigate the preventive effect of two different adhesives on enamel demineralization and compare these adhesives with a conventional one.

Materials and Methods: Fifteen patients requiring the extraction of their first four premolars for orthodontic treatment were included in the study. One premolar was randomly selected, and an antibacterial monomer-containing and fluoride-releasing adhesive (Clearfil Protect Bond, Kuraray Medical, Okayama, Japan) was used for orthodontic bracket bonding. Another premolar was randomly selected, and a fluoride-releasing and recharging orthodontic adhesive (Opal Seal, Ultradent Products, South Jordan, Utah) was used. One premolar was assigned as a control, and a conventional adhesive (Transbond XT, 3M Unitek, Monrovia, Calif) was used. The teeth were extracted after 8 weeks, and the demineralization areas of the 45 extracted teeth were analyzed using microcomputed tomography with software.

Results: There was no significant difference between the white spot lesion (WSL) rates of the adhesives ($P > .05$). The volumes of the WSLs varied from 0 to $0.019349\, \text{mm}^3$. Although Opal Seal showed the smallest lesion volumes, there was no significant difference in volumetric measurements of the lesions among the groups ($P > .05$).

Conclusions: The findings indicated no significant differences between the preventive effects of the adhesives used in this in vivo study over 8 weeks. (Angle Orthod. 2017;87:841–846.)

KEY WORDS: White spot lesions; Microcomputed tomography

INTRODUCTION

Demineralization of the enamel surfaces, or white spot lesions (WSLs), is one of the most common side effects of fixed orthodontic appliances. Irregularities in the teeth cause retention sites in individuals with malocclusion, and bonded orthodontic appliances result in more retention sites. Therefore, retentive properties of the appliances increase plaque accumulation and Streptococcus mutans colonization and give rise to severe cariogenic risk. Several previous studies have shown an increased incidence of WSLs on the labial surfaces of teeth during orthodontic treatment. The prevalence of WSLs associated with fixed orthodontic treatment has varied from 2% to 97%. Preventing rather than treating WSLs must be the first choice of an orthodontist. For this purpose, application of fluoride agents, oral hygiene instructions, and dietary control are recommended. However, in adolescents, ensuring tooth brushing, dietary control, or other oral hygiene applications may be difficult due to poor compliance. Therefore, the most suitable method for preventing WSLs is application of agents that do not require patient cooperation. Various remineralizing agents, such as fluoride and caseinphosphopeptide-stabilized amorphous calcium phosphate nanocomplexes, can help heal or remineralize those small lesions. Additionally, resin-filled sealants or antibacterial fluoride-releasing adhesives have become commercially available for orthodontic bonding, and these adhesives may be useful for reducing demineralization. A glass ionomer adhesive (Opal Seal, Ultradent Products, South Jordan, Utah) and an antibacterial and fluoride-releasing self-etching adhe-
Sive (Clearfil Protect Bond, Kuraray Medical, Okayama, Japan) with high filler content are available. However, limited studies have investigated the clinical effects of these adhesives, and the investigation methods differed.

Recent studies have used microcomputed tomography (microCT) to investigate enamel demineralization. This method gives the researcher the opportunity to calculate the volume of demineralization areas without any damage to the sample surface. Therefore, evaluation of enamel content with microCT has become popular in recent years. The aim of the present in vivo study was to compare the efficiency of an antibacterial fluoride-releasing and fluoride-recharging adhesive with a conventional orthodontic adhesive in preventing enamel demineralization using a quantitative method.

MATERIALS AND METHODS

This study was approved by the regional ethics committee (OMUKAEK 2015/235). The subjects were 15 patients with a mean age of 14.7 ± 1.12 years (5 boys, 10 girls). They were selected according to the following criteria: (1) four first premolar extraction according to an orthodontic treatment plan, (2) no visible enamel demineralization on the labial surfaces of the first premolars, and (3) no restoration or crown anomalies that might affect the enamel surfaces. The sample size was determined using a past study and 15 teeth per group were estimated to give 97.77% power to detect significant differences with a 1.499 effect size and at α = 0.05 significance level. The Cohen’s d statistic was used to calculate the effect size.

Before bracket bonding, all first premolars were cleaned and polished with pumice. In five randomly selected patients, the maxillary right first premolar was bonded using an antibacterial and fluoride-releasing self-etching adhesive (Clearfil Protect Bond, Kuraray Medical), the maxillary left first premolar was bonded with fluoride-releasing and recharging adhesive (Opal Seal, Ultradent Products), and the lower right first premolar was bonded with nonfluoride conventional adhesive as the control (Transbond XT, 3M Unitek, Monrovia, Calif). All bonded first premolars were etched with 37% phosphoric acid according to the manufacturer’s recommendations and then rinsed with water and dried. The same metal brackets (Gemini, 3M Unitek) were bonded to all the teeth. Clearfil and Opal Seal were applied to the teeth according to the manufacturers’ instructions. The quadrants were inverted and the sides bonded with Clearfil, Opal Seal, and Transbond were alternated with each consecutive five patients to reduce any bias. The distributions of the samples according to the groups are shown in Table 1. Excess adhesive was removed with a periodontal probe in each group, and then the adhesive was cured for 10 seconds with an Elipar S10 LED (3M Unitek). The same investigator performed all bonding procedures.

Elastic separation rings were placed around the bracket wings to encourage an increase in plaque accumulation. The patients were recalled 4 weeks after bracket bonding, and plaque accumulation was confirmed. Opal Seal is designed to be reapplied periodically during orthodontic treatment. Shining an ultraviolet black light on the tooth will indicate the presence of the sealant. At the first appointment after bonding, a researcher used a black-light source to

Table 1. Distribution of the Samples According to the Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Maxillary Left Premolar</th>
<th>Maxillary Right Premolar</th>
<th>Mandibular Left Premolar</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearfil Protect Bond</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Opal Seal</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Transbond XT</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>15</td>
</tr>
</tbody>
</table>

Figure 1. View of Opal Seal adhesive on a first premolar under black light (A) just after bracket bonding and (B) at the first appointment after 4 weeks, when a lack of adhesive was seen at the enamel surface.
examine the amount of adhesive remaining on enamel surfaces bonded with Opal Seal adhesive. If there was a lack of adhesive on any part of the enamel surrounding the brackets, new adhesive was added (Figure 1). The patients did not use any other antibacterial or fluoride-containing product during this period. Eight weeks after bracket bonding, all three teeth were extracted at the same time and stored in distilled water until scanning with microCT.

All extracted premolars were cut under the cemento-enamel junction to decrease the volume of the scanned sample; only crowns were scanned. All samples were scanned with a SkyScan 1172 microCT system in the same position using the same procedure. Digital sectional images were obtained using 100 kV accelerating voltage, 100 mA beam current, and 0.5 mm aluminum filter. Each scanning procedure was conducted using 11-megapixel cameras over 50–60 minutes. The images were scanned with a voxel size of 2.56 \( \mu \text{m} \), and 800–900 cross-sectional images were recorded in DICOM (Digital Imaging and Communications in Medicine) file format.

The volumes and depths of the WSLs were calculated using ImageJ software (ImageJ 1.43, Wayne Rasband, National Institutes of Health, Bethesda, Md). First, DICOM images were transformed to grayscale, and subsurface lesions resembling craters were detected by the investigator (Figure 2A). After that, the demineralized enamel was isolated by adjusting thresholds (lower threshold: 0; upper threshold: 50). The edges of the WSL crater were combined using a convex hull module of the software, and the voxel number of the lesions under this line was measured (Figure 2B). This value was converted to cubic millimeters (mm\(^3\)) by multiplying the voxel size of the microCT images by the voxel number.

### Statistical Analysis

The Shapiro–Wilk normality test was applied to volumetric measurements, and the Kruskal–Wallis test was used to compare the effects of adhesive types on WSL volume. The \( \chi^2 \) test was used to identify if there were any differences among the rates of WSLs observed in the Clearfil, Opal Seal, and control groups (Transbond XT). All statistical analyses were performed using SPSS statistical software (SPSS for Mac; SPSS version 20.0, Chicago, Ill). The level of significance was set at \( P < .05 \).

### RESULTS

A total of 45 teeth were evaluated; WSLs were observed in microCT images of 30 teeth (66.6\%). The distributions of the rates of WSLs according to the groups are shown in Table 2. All WSLs measured were located around the brackets. There was no significant difference among the WSL rates of the Clearfil, Opal Seal, and Transbond XT adhesives (\( P > .05 \)).

Figure 2. (A) Detection of a white spot lesion (WSL) in grayscale mode. (B) Measurement of the volume and depth of the lesion.
volumes of the WSLs varied from 0 to 0.019349 mm³. The volumetric measurements of all patients and teeth, according to the groups, are shown in Figure 3. The WSL volume was lower in the Opal Seal group than in the Clearfil and Transbond XT groups, but there was no significant difference among the groups (Table 3). Also, there was no statistically significant difference in depth of the WSLs (Table 3).

**DISCUSSION**

Preventing WSLs during orthodontic treatment is quite important in order to obtain more esthetic results after debonding of brackets. Recently, studies have focused on WSLs because of the increase in the number of agents that can be used to prevent these lesions. The aim of this in vivo study was to compare the efficiency of two remineralizing agents in preventing enamel demineralization using microCT analysis.

There are different methods for assessing demineralization of enamel surfaces. Chemical, radiographic, and microhardness methods were used in previous studies. MicroCT was used to evaluate the WSLs in the present study. This method allows three-dimensional analysis of samples in order to calculate the volume of the lesions, and similar methods were also used in some past studies. The surfaces of the samples were not damaged during scanning, giving the investigator the opportunity to conduct repeated scanning and sensitivity measurements. However, the costs of the technique, the long scanning time of the samples, and the prolonged time needed for analyzing the data are negative aspects of microCT.

In the present study, WSLs occurred in 66.66% of the samples. The incidence of WSLs was different from the findings of past studies. Tufekci et al. examined lesion formation visually and found an overall WSL incidence of 38%. Some other studies reported incidences of 36% and 50% at least one tooth, but they used methods different from those of the current study to examine the incidence of WSLs, such as postorthodontic intraoral photographs. The results of this study exhibited higher incidence than past studies. This difference may be explained by the methodologic difference. The microCT method enables the investigator to note small lesions that are not easy to observe visually.

Antibacterial and fluoride-releasing adhesive was used in some previous studies to prevent enamel demineralization. Uysal et al. evaluated the effect of this adhesive on reducing enamel demineralization and compared it with a conventional adhesive by using cross-sectional microhardness; they found that Clearfil was significantly more efficient. The current results did not confirm those findings. In the current study, although the Clearfil group showed lower WSL volumes than the control group, the lesions were deeper than in the other two groups. However, these differences were not statistically significant. Uysal et al. extracted the teeth 30 days after bonding. This observation time was shorter than in the current study, and a longer bracket presence in the oral cavity may change the results because the amount of daily fluoride released has been shown to undergo a rapid decrease after 3 days and the amount of fluoride released continues to decrease over time. Another previous study that created in vitro caries by pH cycling showed no difference between Clearfil and Transbond XT, but the test conditions were different from those of the current, in vivo study. To prevent enamel demineralization around brackets, minimum levels of fluoride may be needed but with continuous application. The amount of fluoride shown to be released by Clearfil adhesive was 450 μg/g in 1 month, but there is no precise information in the literature on the minimum level of fluoride required to prevent WSLs during orthodontic treatment.

Opal Seal is highly filled with glass ionomer particles (38%) and nanofillers that are released during orthodontic treatment. Under black light, the sealant illuminates if there is still fluoride in its content. After 8 weeks, it has been shown that 50% of the Opal Seal was removed from the tooth surface. In the present study, primer loss was not evaluated, but the fluoride property

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**Table 2.** Prevalence of White Spot Lesions of Adhesives Used in This Study

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearfil Protect Bond</td>
<td>9 (60%)</td>
<td>6 (40%)</td>
</tr>
<tr>
<td>Opal Seal</td>
<td>9 (60%)</td>
<td>6 (40%)</td>
</tr>
<tr>
<td>Transbond XT</td>
<td>12 (80%)</td>
<td>3 (20%)</td>
</tr>
</tbody>
</table>

*χ² = 1.800; P = .407.*

**Figure 3.** Volume of the white spot lesions in each sample. tb: Transbond group; cf: Clearfil group; os: Opal Seal group.
was examined using black light 4 weeks after bracket bonding. If any absence of adhesive or a decrease in illumination was observed, additional material was added and the adhesive was recharged. In this study, new adhesive was applied during the experimental period for only five teeth. The volumes of the WSLs in the Clearfil, Opal Seal, and Transbond XT groups were 0.003410 mm$^3$, 0.002552 mm$^3$, and 0.005032 mm$^3$, respectively. The Opal Seal group showed the lowest WSL volume, but there was no significant difference among the groups. The results of the study may have been affected by the split-mouth design. In the literature, previous studies also used split-mouth designs to investigate WSL formation around orthodontic brackets. Although this design provides bias control, it may be disadvantageous for fluoride-containing materials. Benson and Lessaffre et al. emphasized that there is a possibility of cross-contamination from one side to the other. The intent was to decrease the effect of cross-contamination by bonding only one tooth with a bracket in each quadrant.

In this study, the brackets remained in the oral cavity for 8 weeks. This short duration does not simulate the total orthodontic treatment time. However, studies have reported that enamel demineralization could occur in less time. In a previous study, measurable demineralization occurred around the brackets on extracted teeth in 30 days after bonding. According to Törnlakovic et al., 70% of the teeth developed clinically visible WSLs after 7 weeks. Nevertheless, long-term clinical studies would be of more benefit to investigate the clinical effects of fluoride-releasing and antibacterial materials in the prevention of WSLs.

**CONCLUSIONS**

- No significant differences in the volume and depth of WSLs were found among the teeth bonded using Clearfil, Opal Seal, and Transbond XT adhesives over 8 weeks.
- Long-term clinical studies that investigate the effect of preventing demineralization of these adhesives may produce different results.

**ACKNOWLEDGMENT**

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**REFERENCES**


