Optical evaluation of enamel white spot lesions around orthodontic brackets using swept-source optical coherence tomography (SS-OCT): An in vitro study

Pavethynath VELUSAMY1, Yasushi SHIMADA2,3, Zuisei KANNO1, Takashi ONO1 and Junji TAGAMI2

1 Department of Orthodontic Science, Division of Oral Health Sciences, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan
2 Department of Cariology and Operative Dentistry, Division of Oral Health Sciences, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan
3 Department of Operative Dentistry, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8525, Japan

Corresponding author, Zuisei Kanno; E-mail: z.kanno.orts@tmd.ac.jp

The aim of this study was to evaluate in vitro detection of enamel white spot lesions around orthodontic brackets using swept-source optical coherence tomography (SS-OCT). Twenty-four clear orthodontic brackets were bonded onto the enamel surface of bovine incisor specimens using three types of orthodontic resin adhesives: non-fluoridated, fluoridated, and fluoridated light cured. The specimens were subjected to artificial demineralization. SS-OCT images were captured before demineralization and at 24 h and 1 week after demineralization. Lesion depth (LD) was measured and analyzed using Image J software. Results revealed significant increases in LD with time in all three groups. LDs were, however, significantly smaller in the fluoridated adhesive groups than in the non-fluoridated group. In addition, SS-OCT was validated for the detection of micro-leakage and white spot lesions beneath and around the orthodontic brackets.

Keywords: White spot lesion, Optical coherence tomography, Enamel demineralization, Orthodontic adhesives, Orthodontic brackets

INTRODUCTION

Despite recent advances in dental materials, the occurrence of enamel white spot lesions (WSLs) during orthodontic treatment remain a major concern for orthodontists. WSLs can appear within approximately 4 weeks of the start of fixed appliance therapy because of the difficulty in removing plaque around orthodontic appliances, which also involves patient compliance. These early incipient subsurface lesions present no cavitation and the enamel surface remains intact, making it difficult to diagnose WSLs during their initial stages.

Early detection of WSLs followed by efforts to arrest or remineralize enamel surface lesions is critical during orthodontic therapy. Fluoride plays a major role in not only preventing demineralization but also in enhancing the remineralization process in the early stages especially in non-compliant patients. To aid these patients, manufacturers have also developed several modifications in fluoride-releasing orthodontic adhesives. Previous studies have reported that these fluoride-releasing adhesives can prevent enamel demineralization and facilitate the remineralization of incipient lesions.

Although several methods have been used in clinical studies to detect and monitor carious lesions, visual inspection remains the most common diagnostic method; however, it is subject to error. Conventional radiography can be used to detect only cavitated lesions and not early incipient lesions. Other diagnostic methods such as light-induced or laser-stimulated fluorescence and electric conductivity, can detect early incipient lesions, they cannot be used to visualize cross-sectional images of the dental structures and are not as effective in tracking minimal changes in enamel subsurface lesions. Therefore, an alternative diagnostic method is necessary to detect WSLs during orthodontic therapy.

Swept-source optical coherence tomography (SS-OCT) is a noninvasive diagnostic method for obtaining cross-sectional images of internal biological structures. SS-OCT works under the concept of low-coherence interferometry, in which light is projected onto a sample and the backscattered signal intensity from the scattering medium reveals depth-related information about the scattering and reflection of light in the sample. During demineralization there is an increased amount of enamel porosity because of mineral loss, which leads to 2–3 folds increased magnitude of scattered coefficient values in SS-OCT, thereby detecting WSLs.

Nakagawa et al. validated the use of SS-OCT through in vitro evaluation of caries extent in smooth enamel surfaces; the results were compared with visual inspection on sensitivity and confirmed by observation under scanning confocal microscope. Additionally, SS-OCT has higher sensitivity and specificity than radiographic methods and, therefore, has also been used to diagnose proximal caries and non-caries cervical lesions in vivo.

The purpose of the present study was to use SS-OCT as a possible diagnostic tool in the detection of early enamel demineralization around orthodontic brackets and to evaluate optical changes in the surface of
demineralized enamel using three different fluoridated orthodontic resin adhesives.

MATERIALS AND METHODS

Materials
Three resin adhesives were used in this study: 4-META/MMA-tri-n-butylborane (TBB)-based resin self-cure adhesive system without fluoride-releasing ability (Super-Bond, Sun Medical, Moriyama, Japan, group 1); 4-META/MMA-TBB-based fluoride-containing resin self-cure adhesive system (Super-Bond/F3, Sun Medical, group 2); and fluoridated composite resin containing a surface pre-reacted glass-ionomer (S-PRG) filler particle light-cure adhesive system (Beauti Ortho Bond II, Shofu, Kyoto, Japan, group 3, Table 1). Clear plastic lower incisor orthodontic brackets with 0.018-inch slot size (CrystaBrace3, Dentsply-Sankin, Tokyo, Japan) were bonded to the bovine incisor specimens obtained from a local slaughterhouse in Yokohama, Japan. The bracket wings were cut using a low-speed handpiece (NSK, Tokyo, Japan) to obtain better images for analysis using SS-OCT.

Specimen preparation
Twenty-four fresh bovine incisors were cleaned of debris and soft tissue. Enamel blocks (5×7×3 mm³, width×length×depth) were then cut from the incisors using a low-speed diamond saw (Isomet, Beuhler, Lake Bluff, IL, USA) under running water. The outer enamel surface was polished to a mirror finish using wet polishing papers (800, 1000, 1200, 1500 and 2000 grit lapping papers [3M, St. Paul, MN, USA]). SS-OCT uses backscattered reflected light to measure the lesion depth (LD). Because bovine enamel is irregular in shape and may scatter light and lead to measurement errors, the enamel surfaces were flattened using wet polishing papers to maintain uniformity of the signal intensity.

The specimens were randomly divided into three groups according to the adhesive system used. The enamel surfaces were polished with non-fluoridated pumice using a rubber cup, thoroughly rinsed, and air-dried.

Bonding procedures
Super-bond Orthomite and Super-bond F3 Orthomite orthodontic adhesive (SEP+monomer+catalyst+conventional polymer powder, or fluoride-containing polymer powder) were used for groups 1 and 2, respectively. Phosphoric acid (65%; Red activator, Sun Medical) was applied to the enamel surfaces for 20 s using a sponge pellet. An air-water syringe was used to rinse and remove the acid. An air jet was gently applied to dry the enamel surfaces. Appropriate amounts of monomer (Quick Monomer, Sun Medical) and the TBB catalyst (Catalyst V, Sun Medical) were mixed well, and non-fluoridated conventional polymer powder (Polymer Clear, Sun Medical [group 1]) or fluoride-containing polymer powder (Brush-dip F3, Sun Medical [group 2]) was added to the well. Pre-cut clear plastic brackets with a base area of 3.0 mm² were bonded using a brush-dip technique.

The Beauty Ortho Bond II light-cure orthodontic adhesive was used for group 3. Plastic bracket primer was applied to the enamel surface for 10 s, and an air jet was gently applied to the enamel. Adhesive paste (Beauty Ortho Bond II Paste [viscous]) was applied to the base of the bracket and was positioned on the conditioned enamel surface. All excessive adhesive flash (EAF) in the groups was removed around the bracket area using an explorer. Specimens in group 3 were irradiated using a halogen light-curing unit (600 mW/cm²) output (Optilux 501, Kerr, Orange, CA, USA) for 10 s.

Table 1  Orthodontic adhesives used in this study

<table>
<thead>
<tr>
<th>Groups</th>
<th>Material</th>
<th>Components</th>
<th>Composition</th>
<th>Fluoride</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Superbond Orthomite</td>
<td>Red activator</td>
<td>65% Phosphoric acid, PMMA, MMA, 4-META, Tri-n-butylborane</td>
<td>Absent</td>
<td>Sun Medical, Moriyama, Japan</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Polymer Monomer</td>
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<td></td>
<td>Catalyst</td>
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</tr>
<tr>
<td>Group 2</td>
<td>Superbond F3 Orthom</td>
<td>Red activator</td>
<td>65% Phosphoric acid PMMA, MMA, 4-META, Tri-n-butylborane</td>
<td>Present</td>
<td>Sun Medical</td>
</tr>
<tr>
<td></td>
<td>Ortomite</td>
<td>Polymer Monomer</td>
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<td>Catalyst</td>
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<tr>
<td>Group 3</td>
<td>BeautiOrtho Bond II</td>
<td>Primer A</td>
<td>Water, acetone, others, phosphoric acid monomer, carboxylic acid monomer, ethanol, TEGDMA, surface pre-reacted glass ionomer, Bis-GMA, MMA</td>
<td>Present</td>
<td>Shofu, Kyoto, Japan</td>
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<tr>
<td></td>
<td></td>
<td>Primer B</td>
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<tr>
<td></td>
<td></td>
<td>Paste</td>
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</table>

PMMA: polymethyl methacrylate, MMA: methyl methacrylate, 4-META: 4-methacryloyloxyethyl trimellitate anhydride, TEGDMA: triethyleneglycol dimethacrylate, Bis-GMA: bisphenol-A-diglycidyl methacrylate
**Immersion in demineralization solution**
The bonded specimens were immersed individually in a plastic vial containing 10 mL of distilled water at 37°C for 24 h. They were subsequently washed with fresh distilled water and immersed into a separate vial containing buffered demineralization solution (pH 4.5, 2.2 mM CaCl₂, 2.2 mM Na₂HPO₄, and 50 mM acetic acid). The demineralization solution was changed every 24 h.

**OCT System**
The SS-OCT system (IVS-2000, Santec, Komaki, Japan) was used to examine the specimens before demineralization (DeM0), and 1 day (DeM1), and 1 week (DeM1’) after demineralization. This frequency-domain OCT technique can identify the magnitude and coherence of reflected light from the specimen into the depth-profile of the specimen. It incorporates a high-speed frequency and a swept external-cavity laser. The wavelength ranged from 1,260 to 1,360 nm, and was centered at 1,310 nm with a sweep rate of 20 kHz. The optical resolution was 17 µm laterally and 11 µm axially in the air, which corresponds to 7 µm in tissues assuming a refractive index of approximately 1.5.

The sample size for this study was calculated using statistical power analysis. Eight enamel samples were used for each group, and a total of 40 OCT images were captured at five different imaging points for each of the samples. The mean values were then calculated. Five imaging points were chosen on each specimen along its long axis (two marginal locations on both ends and one in the center). SS-OCT examination was performed along the plane at these five points and central cross-section images were obtained. Images were captured after the brackets were bonded and allowing the specimens for a 24 h to set (DeM0). Subsequently, images (DeM1 [1 day] and DeM1’ [1 week]) were also captured at the same points. The demineralized specimen surfaces were gently blot-dried, leaving them moist without any visible water-droplets. The specimens were scanned at the same orientation as accurately as possible. The specimens were tilted 3–5° to prevent reflections from the surface in the image.

A custom code in the image analysis software (Image J, version 1.48, National Institutes of Health, Bethesda, MD, USA) was used to read the raw SS-OCT data. Images from the raw data were increased to a 3:1 scale (5,000×7,481 µm). A threshold plugin was applied and a region of interest (ROI) 100×100 mm (333.33×748.1 µm) was chosen adjacent to the bracket area. The ROI was chosen 50 mm away from the edge of the bracket area considering any backscattered error values from the SS-OCT values. During the bonding procedure, all EAF around the brackets was removed, acknowledging the possibility of some adhesive remnants around the bracket in clinical settings. The samples were examined for EAF using a simple microscope before subjecting them to artificial demineralization to rule out such possibilities.

**Statistical analysis**
Statistical analysis was performed using a spreadsheet (Excel, Microsoft, Redmond, WA, USA) for Macintosh (Apple, Cupertino, CA, USA) version 16.9. The mean LDs of the groups were compared at the 1-day and 1-week demineralization time points (i.e., DeM1 and DeM1’) using independent sample t-tests. Statistical analyses were performed at a 95% level of confidence.

**RESULTS**
The differences in signal intensities due to demineralization in the samples are shown in Fig. 1. The results of this study (Fig. 2) revealed significant increases in LD with increase in time (p<0.05). The non-fluoridated Super-bond adhesive (group 1) exhibited the most amount of demineralization compared with the other two fluoridated groups, both at the 1-day and 1-week time point. Both fluoridated adhesive groups (groups 2 and 3) exhibited an increase in LDs during
Fig. 2 SS-OCT analysis of mean lesion depth.
The demineralization lesion depths (LD in µm) were calculated from the ROI and the mean values were compared between the groups between DeM1 and DeM1’. The values showed significant difference between DeM1 and DeM1’ within each group (p<0.05), indicated by an asterisk mark (*). ROI: region of interest, DeM1: after one day of demineralization, DeM1’: after one week of demineralization. Group 1: Superbond Orthomite, Group 2: Superbond F3 Orthomite, Group 3: BeautiOrtho Bond II.

DeM1 but significantly smaller depths compared with the non-fluoridated adhesive group (Fig. 3). There was no significant difference between the two fluoridated adhesives at the 1-week time point (i.e., DeM1’).

DISCUSSION
Under OCT, sound enamel is almost transparent at the near infrared region, and demineralization causes increase in signal attenuation17,19). This enables SS-OCT to detect enamel demineralization as a bright lesion with increased backscattered signal. Demineralization of the enamel surface is caused by mineral loss, which creates a large number of micro-interfaces which lead to an increase in backscattering (reflection) of light20). This increases the signal intensity and causes increased brightness in the corresponding OCT image (Fig. 1), measured as LD. Clinically, this gives the characteristic chalky white appearance of the enamel surface, which gave rise to the term WSLs.

There is a visual boundary between the dark and bright grey-scale OCT image that was reported to be associated with LD21). This is because the effective imaging capability of OCT is only a few millimeters from the surface; therefore, the deeper the lesion, the weaker the signal strength.

Using SS-OCT, raw data from images were acquired for each sample at the five different locations. The area of
demineralization for the ROI was subsequently calculated from the same DeM1 and DeM1’ images. Although SS-OCT images were captured before demineralization (DeM0), they were assumed to be zero because the samples were not subjected to demineralization and, therefore, no calculations were performed for DeM0. The values cited in Figs. 2 and 3 are the optical LD values (in µm), which were calculated from the area of demineralization by dividing them by the width of the ROI (333.3 µm).

The refractive index of enamel \(^22\) (i.e., 1.631) must be considered while interpreting the real LD from the optical depth values. Group 1 samples exhibited considerable increases in LD due to the absence of fluoride content, which, to a certain extent, aided in inhibiting demineralization progress around the bracket area in the other two groups. Although the fluoridated groups did not exhibit appreciable differences in demineralization at DeM1’, there was a significant difference at DeM1, which illustrated that the significant effect of fluoride within the 1-day time period. Of the three groups, group 3 exhibited the least amount of demineralization at day 1, which may have been due to the fact that the Beauty Ortho Bond II bonding system involves an increased fluoride recharging capability was not examined in the present investigation, previous study\(^23,24\) have reported the property of being fluoride rechargeable. Although fluoride recharging capability was not examined in the present investigation, previous study\(^23,24\) have reported that adhesives containing S-PRG filler particles can be used as an excellent fluoride reservoir to prevent demineralization.

The increased amount of demineralization at DeM1 in group 2 —compared with group 3— may be due to differences in enamel conditioning. In group 2, 65% phosphoric acid was used, which may have conditioned the cut bovine enamel for deeper penetration of etchant, thereby creating large porous areas for demineralization. In group 3, this was not the case because enamel conditioning was performed using a primer without etching\(^25\).

The results of this study with regard to the appearance of WSLs are in general agreement with those of previous studies that used polarization sensitive-OCT or SS-OCT for the detection of carious lesions\(^16,17,21\). In this study, we also found that SS-OCT was capable of detecting micro-leakage beneath the base of the bracket. This frequently occurs in clinical situations due to deficient or improper sealing of orthodontic adhesives while bonding the brackets, which in turn could lead to caries under the bracket if left unchecked. This study also confirmed that SS-OCT was capable of detecting micro-leakage gaps at the bracket-tooth interface. Further clinical studies in monitoring WSLs using SS-OCT may provide more insight about the diagnostic potential of this noninvasive imaging system.

**CONCLUSIONS**

In this study, we were able to optically evaluate enamel WSLs around the orthodontic bracket using SS-OCT and demonstrate that fluoride-containing orthodontic adhesives were able to inhibit demineralization for a certain period of time and may provide sufficient anti-demineralization effects around the enamel area surrounding orthodontic brackets. SS-OCT can also be used in orthodontics as a tool for detecting WSLs during its initial stages, which can be used as an effective preventive measure that can save considerable treatment time for patients.

**REFERENCES**


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