Assessment of occlusal fissure depth and sealant penetration using optical coherence tomography

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Swept source optical coherence tomography (SS-OCT) is an interferometric imaging technique. This study aimed to assess SS-OCT as a diagnostic tool for the evaluation of occlusal fissure depth and sealant penetration into fissure. Seventy investigation sites of occlusal fissures without visible evidence of caries were categorized into four groups (smooth, shallow, intermediate, and deep fissures). Categorization utilized two methods: 1) visually, using a computer screen, and 2) using SS-OCT images. After sealant placement, penetration was observed in SS-OCT. The results obtained from SS-OCT and visual inspections were compared with those of confocal laser scanning microscope (CLSM). The diagnostic power of SS-OCT was higher than that of visual inspection for fissure depth. Additionally, clear cross-sectional images of sealant penetration into fissures were observed with SS-OCT. SS-OCT can be used to evaluate fissure depth and monitor sealant penetration.

Keywords: Swept source optical coherence tomography (SS-OCT), Occlusal fissure, Fissure sealant, Confocal laser scanning microscope (CLSM)

INTRODUCTION

Caries involving the occlusal surfaces account for almost 60% of the total caries experience in the permanent dentition of children and adolescents. Since occlusal caries account for such a large percentage of total caries, dentists are often confronted with the task of preventing fissure caries. Tooth surfaces with pits and fissures are particularly vulnerable to caries development due to the complexity of their morphology. The complex anatomical characteristics of fissures may also be an obstacle to caries prevention and remineralization, as plaque removal and penetration of fluoride and bactericidal solutions are hampered within fissures. Previous studies have indicated that fissure morphology (shape, depth and narrowness) was related to caries susceptibility. Microscopic investigations have shown that occlusal fissures that clinically appear round and shallow occasionally harbor deep grooves which are occluded with debris and microorganisms.

An effective treatment for the prevention of occlusal caries is the use of pit and fissure sealants. The cariostatic properties of sealants are attributed to formation of a physical barrier over the pits and fissures. Although pit and fissure sealants have been shown to be effective for caries prevention, sealed surfaces require careful assessment and monitoring for caries detection over the long term; examiners can underestimate the severity of lesions detected after sealing compared with their assessment prior to sealing.

Generally, clinicians have used a visual-tactile examination technique using a sharp explorer for assessing fissure depth and shape. However, probing has the potential to inflict iatrogenic damage.

Optical coherence tomography (OCT) is a noninvasive imaging technique that produces high-resolution, cross-sectional images of biological tissue at a micrometer scale. Recently, OCT technology has been significantly advanced by the development of spectral discrimination (SD) techniques, which provide a substantial increase in sensitivity over earlier systems. The swept source optical coherence tomography (SS-OCT) method is one of these SD developments and uses a wavelength-tuned near-infrared laser as the light source, thus providing improved imaging resolution and scanning speed. In laboratory studies, it was reported that SS-OCT has the ability to differentiate between carious and sound tissues in both primary and permanent teeth, estimate the lesion depth in demineralized tissues, observe gaps and defects in dental restorations, and detect tooth cracks.

The aim of this study was to assess SS-OCT as a diagnostic tool for the detection of occlusal fissure depth. The results of SS-OCT in vitro were compared with those of visual inspection; direct observation of the specimens under confocal laser scanning microscope (CLSM) served...
as the validation method. Moreover, the penetration of sealant into the fissure was monitored by SS-OCT.

MATERIALS AND METHODS

SS-OCT system
The SS-OCT system (IVS-2000, Santec, Komaki, Japan) used in this study is a frequency (Fourier) domain OCT technique that measures the magnitude and time delay of reflected light in order to construct a depth profile. This SS-OCT system incorporates a high-speed frequency swept external cavity laser, of which the hand-held probe power is less than 5 mW, in accordance with the safety limits of the American National Standards Institute. The center wavelength is 1,319 nm with a scan range of 112 nm at a 20 kHz sweep rate. The spectral bandwidth of the laser exceeds 100 nm centered at 1,319 nm at a 20 kHz sweep rate. Axial resolution of this system in air is 11 µm, which corresponds to 7 µm within dental tissue having a refractive index of about 1.521). The lateral resolution of this system is 17 µm, which is determined by the objective lens and beam diameter inside the probe. The cross-sectional scans were acquired at 2,001×1,019 pixels within 0.3 s.

Tooth preparation
Thirty-two extracted human premolars that had no visible evidence of caries were stored at 4°C in saline containing a few thymol crystals and used for this in vitro study. The use of these teeth was approved by the Institutional Review Board of Tokyo Medical and Dental University (Nos. 578 and 599). The teeth were mounted on 10×10 mm methyl-methacrylate resin (Unifast III, GC, Tokyo, Japan) blocks after root resection, 70 locations on the occlusal fissures were arbitrarily selected from the 32 premolars and designated by a marker pen as the inspection sites. Three experienced dentists (YS, AS, and YN) participated in this study as examiners, each of whom had over 5 years of clinical experience in dentistry. In order to reach a consensus on diagnostic criteria, the reference investigator (SI) discussed the SS-OCT imaging with the three examiners in a one-hour session using sample investigation sites that were not included in the main study. The examiners were unable to see the selection procedure for investigation sites and were blind to other examiners’ results. Visual inspection and SS-OCT evaluations were performed in separate sessions after shuffling the order of the cases to ensure there was no interference from previous observations.

Visual inspection (VI) of fissure depth
After cleaning and drying the occlusal surface of the tooth, a photograph was taken of the investigation site using a digital camera (EOS Kiss×4, Canon, Tokyo, Japan) and the image displayed on a computer screen at an approximate magnification of 10×. The investigation sites were designated by a blue line on the digital image. All examiners classified the investigation sites and scored the fissure depths as follows:

Smooth fissure (score 0): The fissure base shows no cleaving between the cuspal inclines.
Shallow fissure (score 1): The base of the fissure cleft is less than one-third of the enamel thickness.
Intermediate fissure (score 2): The base of the fissure cleft is deeper than one-third but less than two-thirds of the enamel thickness.
Deep fissure (score 3): The fissure is deeper than two-thirds of enamel thickness, near the dentinoenamel junction.

SS-OCT observation of fissure depth
The hand-held scanning probe connected to the SS-OCT system was set at a fixed distance over the occlusal fissures, with the scanning beam oriented at 90° with respect to the tooth occlusal plane of the tooth. The SS-OCT images were obtained as buccolingual cross-sections of the same 70 selected sites, where the VI had been performed. The SS-OCT images of the investigation sites were classified and scored by the examiners following the same scoring criteria as explained for VI; scores 0 to 3.

Sealant application and SS-OCT imaging of sealant penetration
The sealant material (Teethmate F-1, Kuraray Noritake Dental, Tokyo, Japan) was placed by the reference operator (SI), according to the manufacturer’s instructions. A clear shade of sealant material was used. First, the fissures were cleaned without prophylaxis paste using a brush cone attached to a low-speed handpiece and thoroughly washed with a high-pressure stream of water and air spray. The fissures were then etched with a 40% phosphoric acid gel (K-Etchant gel, Kuraray Noritake Dental) for 40 s, rinsed thoroughly with water, and gently air dried until the occlusal surfaces appeared frosty. The applicator nozzle of the sealant was placed against the fissures, and the barrel of the container was gently squeezed for precise sealant placement. A sealant applicator instrument (Sealant applicator, GC) was used to carefully spread the sealant if necessary. The
sealant material was light cured for 20 s with a halogen light curing unit (OptiLux 501, Kerr, Orange, CA) with an output of 600 mW/mm².

Following sealant placement, observations were performed with SS-OCT to evaluate the completeness of sealant penetration into each fissure depth.

**CLSM observation**
Validation of the measurements by SS-OCT and VI was performed by direct observation of the sectioned
Fig. 5 Representative images of a deep fissure (score 3).
(a) In the photograph, the investigation site for VI and SS-OCT assessment is indicated by the blue line. (b) SS-OCT image prior to sealant placement at the location in (a). The dentin-enamel junction (DEJ) is indicated by an arrow head. (c) SS-OCT image after sealant placement at the location in (a). Lack of complete sealant penetration at the base of the fissure could be observed as a dark zone (arrow). (d) Observation of the cross-sectioned surface by CLSM. The lack of sealant penetration into deep fissure was confirmed (arrow).

Fig. 6 Representative images of complete sealant penetration.
(a) In the photograph, the investigation site for VI and SS-OCT assessment is indicated by the blue line. (b) SS-OCT image prior to sealant placement at the investigation site. The dentin-enamel junction (DEJ) is indicated by an arrowhead. The demineralized area was detected as a bright area around the fissure walls and bottom (arrows). (c) SS-OCT image after sealant placement at the same location as in (a) and (b). The complete sealant penetration into the base of the fissure was observed as homogeneous brightness in gray scale. After sealant placement, the demineralized area was detected more clearly as the bright area (arrows). (d) Observation of the cross-sectioned surface by CLSM. The CLSM image correlated well with the SS-OCT image.

Fig. 7 Representative images of incomplete sealant penetration.
(a) In the photograph, the investigation site for VI and SS-OCT assessment is indicated by the blue line. (b) SS-OCT image prior to sealant placement at the investigation site. The dentin-enamel junction (DEJ) is indicated by an arrowhead. A light scattering bright zone was observed in the fissure (arrow). (c) SS-OCT image after sealant placement at the same location as in (a) and (b). After sealant placement, a light scattering bright area was observed in the fissures (arrow). (d) Observation of the cross-sectioned surface by CLSM. The presence of crystalline deposition in the fissure was observed. Sealant penetration into the base of the fissure was disturbed by the presence of crystalline deposition. The CLSM image correlated well with the SS-OCT image.

Fissures using CLSM (1LM21H/W; Lasertec, Yokohama, Japan). For this purpose, the teeth were cross cut in the buccolingual direction at the marked investigation sites using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) and slightly polished under running water with wet silicon carbide papers and diamond paste having particle sizes down to 3 µm. The same location as the slice of SS-OCT cross sectional images was observed with CLSM at a magnification of 1,250×. In total, 70 investigation sites were evaluated in terms
Table 1  The sensitivity, specificity and Spearman’s correlation coefficient values for determining the occlusal fissure depth between the detection methods and CLSM with the respective 95% confidence interval

<table>
<thead>
<tr>
<th>Fissure depth</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Spearman’s correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth fissures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>0.33</td>
<td>0.78</td>
<td>0.08 (p&gt;0.05)</td>
</tr>
<tr>
<td>SS-OCT</td>
<td>1.00</td>
<td>0.98</td>
<td>0.88 (p&lt;0.05)*</td>
</tr>
<tr>
<td>Shallow fissures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>0.31</td>
<td>0.52</td>
<td>0.02 (p&gt;0.05)</td>
</tr>
<tr>
<td>SS-OCT</td>
<td>0.75</td>
<td>0.75</td>
<td>0.50 (p&lt;0.05)*</td>
</tr>
<tr>
<td>Intermediate fissures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>0.26</td>
<td>0.81</td>
<td>0.13 (p&gt;0.05)</td>
</tr>
<tr>
<td>SS-OCT</td>
<td>0.67</td>
<td>0.81</td>
<td>0.43 (p&lt;0.05)*</td>
</tr>
<tr>
<td>Deep fissures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>0.03</td>
<td>0.93</td>
<td>0.09 (p&gt;0.05)</td>
</tr>
<tr>
<td>SS-OCT</td>
<td>0.38</td>
<td>0.94</td>
<td>0.40 (p&lt;0.05)*</td>
</tr>
</tbody>
</table>

* Indicates a significant difference (p<0.05).

Table 2  The sensitivity, specificity and Spearman’s correlation coefficient values for assessing sealant penetration between SS-OCT and CLSM with the respective 95% confidence interval

<table>
<thead>
<tr>
<th>Fissure depth</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Spearman’s correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth fissures</td>
<td>1.00</td>
<td>1.00</td>
<td>0.65 (p&lt;0.05)*</td>
</tr>
<tr>
<td>Shallow fissures</td>
<td>0.78</td>
<td>0.67</td>
<td>0.52 (p&lt;0.05)*</td>
</tr>
<tr>
<td>Intermediate fissures</td>
<td></td>
<td>0.73</td>
<td>0.88 (p&lt;0.05)*</td>
</tr>
<tr>
<td>Deep fissures</td>
<td>—</td>
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<td>—</td>
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</tbody>
</table>

* Indicates a significant difference (p<0.05).

of fissure depth and sealant penetration according to the previously described criteria.

Statistical analysis
Sensitivity and specificity indices for each of the four fissure depth scores (smooth, shallow, intermediate, and deep) were calculated from the scores determined by VI and SS-OCT by each examiner. Sensitivity and specificity were also calculated from the resulting completeness of sealant penetration into each fissure. The representative sensitivity and specificity were calculated by averaging the three examiners’ results in each category. The strength of the association between each method and the CLSM evaluation was analyzed by Spearman’s correlation coefficient. Statistical analysis was performed at a significance level of α=0.05 with the statistical software package (SPSS Version 19 for Windows, SPSS, Chicago, IL, USA).

RESULTS
Distributions of the fissure depth scores are presented in a histogram in Fig. 1. After obtaining the confirmatory CLSM images, the 70 investigation sites were classified: 5 smooth fissures without a cleft (score 0), 17 shallow fissures (score 1), 35 intermediate fissures (score 2) and 13 deep fissures (score 3). Representative images of each fissure depth score and sealant penetration are shown in Figs. 2–5. Figs. 6 and 7 show representative images of complete and incomplete sealant penetration, respectively.

The results and values for sensitivity, specificity and Spearman’s correlation coefficients for the determination of occlusal fissure depth are presented in Table 1. The sensitivity values of SS-OCT were 1.00 for smooth fissures, 0.75 for shallow fissures, 0.67 for intermediate fissures, and 0.38 for deep fissures, whereas the sensitivity values of VI were 0.33, 0.31, 0.26, and 0.03,
respectively. The sensitivity values of SS-OCT were higher than those of VI. The specificity values of SS-OCT were 0.98 for smooth fissures, 0.75 for shallow fissures, 0.81 for intermediate fissures, and 0.94 for deep fissures, whereas the specificity values of VI were 0.78, 0.52, 0.81, and 0.93, respectively. The specificity values of SS-OCT were also higher than those of VI for smooth and shallow fissures. The Spearman’s correlation coefficient values of SS-OCT indicated significant differences for all fissure depths (p<0.05), whereas the Spearman’s correlation coefficient values of VI did not indicate significant differences for all fissure depths (p>0.05).

The values of sensitivity, specificity and Spearman’s correlation coefficients for the assessment (complete versus incomplete) of sealant penetration into each fissure depth as evaluated by SS-OCT are presented in Table 2. The sensitivity values were 1.00 for complete penetration of sealant into smooth fissures, 0.78 for complete penetration into shallow fissures, and 0.73 for complete penetration into intermediate fissures. The specificity values were 1.00, 0.67, and 0.88, respectively. The Spearman’s correlation coefficient values for assessing sealant penetration using SS-OCT indicated significant differences for all fissure depths (p<0.05). In the CLSM observation, incomplete penetration of sealants into the deep fissures was frequently observed. The value for complete penetration of sealant into deep fissures was not reported due to lack of cases of complete sealant penetration into deep fissures.

Table 3 shows the distribution of complete or incomplete penetration of sealant into each fissure depth. In this study, sealant completely penetrated into all smooth fissures and nearly all shallow fissures. However, it was difficult for fissure sealant to penetrate to the base of deep fissures completely.

**DISCUSSION**

In this *in vitro* study, SS-OCT was used to assess the depth of occlusal fissures and the completeness of sealant penetration. This noninvasive technique was capable of providing clear cross-sectional images of occlusal fissures (Figs. 2–5). The sensitivity of fissure depth evaluation using SS-OCT was higher than those of VI for all fissure depths. The specificity of fissure depth evaluation using SS-OCT was higher than those of VI for smooth and shallow fissure depths. Spearman’s correlation coefficients showed that SS-OCT was associated with CLSM, whereas VI was not associated with CLSM. However, signal degradation increased when assessing deeper fissures; therefore a decrease was observed in the sensitivity and the Spearman’s correlation coefficient values of SS-OCT (Table 1).

SS-OCT was also capable of assessing sealant penetration into occlusal fissures (Figs. 2–7). In smooth fissures, all investigation sites showed complete penetration to the fissure base with both SS-OCT and CLSM. Shallow fissure showed 15 complete penetration from 17 investigation sites, whereas intermediate fissure showed 11 complete penetration from 35 investigation sites, and deep fissure showed no complete penetration in 13 sites (Table 3). SS-OCT revealed complete penetration as homogeneous brightness extending into the fissure base in a gray scale image (Figs. 1–4 and 6).

In the case of intermediate and deep fissures, most of the grooves were filled with a light scattering deposition in SS-OCT, indicating sealant did not completely penetrate into the fissures, leaving unfilled zones (Fig. 7). CLSM observations also confirmed the presence of crystalline deposition plugs in the intermediate and deep fissures. These depositions were observed in SS-OCT as distinct bright zones with clear borders distinct from the enamel surface. Demineralized enamel was depicted as a bright zone without a clear border from the enamel; therefore, the crystalline deposition plugs were distinguished from enamel demineralization in SS-OCT (Fig. 6). Interestingly, the presence of these depositions was revealed with greater contrast after the sealant application. The unfilled zone beneath the deposition displayed as a dark zone with lack of signal (Fig. 5). Consequently, SS-OCT could discriminate complete and incomplete sealant penetration based on the presence of both heterogeneous brightness and dark zones when sealant penetration was incomplete (Figs. 5 and 7).

This study shows that the deep fissures were incompletely filled with sealant, while the smooth and shallow fissures were almost completely filled (Table 3). Based on SS-OCT images and confirmatory CLSM observations, the results of current study confirmed that penetration of sealants to the base of fissures occurred...
more frequently in the shallow fissures compared with deeper fissures. Deep fissures are more difficult to clean, etch and dry, thus affecting the ability of the sealant resin to obturate these fissures. CLSM observation revealed that in those cases where the fissure did not appear clearly with SS-OCT regardless of imaging orientation, residual debris or calculus-like deposits in the fissure had in fact blocked complete penetration of sealant into the deeper portion. This also explains why the SS-OCT laser beam could not initially reach the depth of fissure. However, an intimate adaptation of the sealant to the etched enamel could reduce the surface scattering and improve visibility of fissure depth.

Apart from physical barriers discussed above, the lack of complete sealant penetration into deep, clean fissures may partly be explained by material-related factors and procedural issues, such as entrapment of air bubbles while dispensing the sealant flowable resin composite. It has been suggested that these air bubbles may either be present in the syringe during production or form while the material is dispensed from the nozzle tip attached to the container syringe. Another interesting aspect of SS-OCT imaging is that these bubbles can be detected and characterized after, or even prior to, polymerization of the resin, which allows the operator to manipulate or replace the defective sealant immediately.

The capability of the SS-OCT system to image depth is also limited due to technical obstacles such as light attenuation through the structure. Since the laser light of near-infrared at a 1,300-nm wavelength can penetrate with little attenuation through the air and sound enamel, we could obtain clear images of smooth fissures and shallow fissures. Imaging penetration and attenuation of a signal can be influenced by the varying angle of incidence when occlusal surfaces are scanned. The strong reflection at the tooth surface can be a confounding factor. Demineralization in the fissures typically strongly attenuates the OCT signal, preventing an accurate depth determination of the lesion. Birefringence is particularly problematic in conventional OCT systems because it produces artifacts, such as banding, that confound early caries diagnosis. Therefore, wall inclination with respect to OCT beam orientation and appropriate focusing of the SS-OCT beam affect imaging. In this study, the axial orientation and distance from the probe were adjusted for each tooth during SS-OCT scan to ensure an optimal fissure depth imaging. However, because of a more complex structure of deep occlusal fissures than that of smooth and shallow fissures, it was difficult to obtain a clear image of the deep fissures in some cases.

It was reported that the composition of resin affects the OCT light attenuation. In this study, a clear sealant was used to ensure lower scattering of light through the sealant and better SS-OCT imaging depth, as reported previously. This could explain the excellent diagnostic power of SS-OCT for the assessment of sealant penetration depth. However, clinicians may prefer opaque or colored shades of sealants, as it is easier to see the sealant during application and to assess retention at later time intervals when compared with a clear sealant. The opaque shades contain an optical opacifier, titanium dioxide, which strongly attenuates near-IR light. If OCT is to become a standard clinical imaging technique for dentistry, OCT imaging depth should be a consideration in the formulation of new dental materials by manufacturers. The situation may be comparable to efforts made in the past to add radiopaque substances to materials to improve X-ray imaging.

It is noteworthy that although the selected teeth for this study did not exhibit clinical indications of caries in the occlusal surfaces, SS-OCT could frequently identify demineralized lesions at walls and/or in the base of fissures, which appeared as enamel regions with increased backscattering (Figs. 3, 4, and 6). Several studies show that the infrared region from 780 to 1,550 nm (especially the wavelength 1,310 nm) can offer great potential for the optical imaging of sound enamel because of weak scattering and absorption in this region. In the lesion, porosity forms because of partial dissolution of individual mineral crystals. Such small pores act as scattering centers and strongly scatter near-infrared light. The magnitude of the scattering coefficient of enamel increases in proportion to demineralization, and provides high contrast in OCT images, because the demineralization of enamel results from an increase in backscattered light from the lesion area.

This study shows that non-invasive SS-OCT provides significant information to clinicians for detecting and monitoring demineralization before and after sealant placement. A previous study indicates that fissure anatomy is a significant factor for sealant microleakage and penetration ability. However, it is not clearly demonstrated if the increased depth of sealant penetration will result in greater sealant retention or caries prevention in a clinical treatment. SS-OCT must be an adjunct method to increase diagnostic accuracy and to improve monitoring assessments, which will show the relation between sealant penetration depth and caries prevention in clinical dentistry.

CONCLUSION

SS-OCT can accurately reveal occlusal fissure depth and sealant penetration into fissures. The values of sensitivity, specificity and Spearman’s correlation coefficient obtained with SS-OCT detection were superior to those of VI results. Sealant penetration was affected by fissure depth; therefore, SS-OCT is a beneficial technique with which to assess fissure depth and monitor sealant application.

ACKNOWLEDGMENTS

This research was supported, in part, by a Grant-in-Aid for Scientific Research (No. 24592861) from the Japan Society for the Promotion of Science (JSPS); and a Research Grant for Longevity Sciences (21A-8) from the Ministry of Health, Labor and Welfare.
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