INTRODUCTION

Resin-based composites have gained increasing popularity in recent years due to their appealing aesthetic characteristics, especially with the development of adhesive systems. The formation of hybrid layer at the interface is essential to achieve the bond, that requires an infiltration of adhesive resin monomers to the demineralized zone and proper polymerization of the resin monomers, leading to protecting of exposed collagen network, resulting in a stable adhesive layer. In addition, the dentin adhesion based on the micromechanical retention induced by resin tags, which are formed due to resin infiltration into demineralized dentin. However, resin-based composites often experience shrinkage during the polymerization process, creating gaps at the resin-tooth interface. Gap formation around composite restorations is influenced by the cavity preparation method, composite material composition, and application site. Evaluation of gap formation at the interfaces of a two-step self-etching adhesive with/without pre-etching was performed using swept-source optical coherence tomography (SS-OCT). Round cavities were prepared in bovine incisors at the middle (MC) and cervical (CC) thirds of the crown and the cervical third of the root (CR). Clearfil SE bond was directly applied to one group (SE) and another (PA) was pretreated with K-etchant gel. Following restoration by flowable composite resin, the teeth were thermally challenged and stored for 2 months. Interfacial gaps observed in the cross-sectional OCT images were analyzed and the bottom cavities exhibited increased gaps compared to the margin and dentin-enamel junction (DEJ). The CR site had a larger gap than at MC and CC in the SE group. DEJ separation at the MC was significantly smaller than that at CC in both groups. Therefore, gap formation depends on the cavity region, location, and bonding protocol.

Keywords: Self-etch adhesive, SS-OCT, Gap, Bottom, Dentin-enamel junction
regions of the cavity, (2) no difference in gap formation at different sites of application, and (3) pre-etching treatment did not influence gap formation of the cavities bonded with self-etch adhesive.

**MATERIALS AND METHODS**

**SS-OCT system**

SS-OCT (IVS-2000, Santec, Komaki, Japan) is a frequency domain OCT system which was integrated with a low-coherence near-infrared light source. Near-infrared light is a high sweeping laser that repetitively sweeps from 1,260 to 1,360 nm (centered at 1,310 nm) with a 20 kHz rate. The axial resolution of the system in air is 12 µm, corresponding to 7 µm within dental tissue with a refractive index of approximately 1.5[9]. The lateral resolution of the system is 17 µm, which was determined by the objective lens and probe beam diameter. The probe in this system, which was connected to the interferometer, has a power of less than 5 mW, which is within the safety limits of the American National Standards Institute. The laser beam scans the object in the X and Z dimensions and the backscattered light carrying information from each point in the sample is returned to the system, digitized as a function of time, and analyzed in the Fourier domain to form a depth-resolving scan (A-scan) at each point to create a cross-sectional B-scan image.

**Specimen preparation**

The materials used in this study are listed in Table 1. A total of 20 freshly extracted bovine incisors without any evidence of enamel cracks, were polished with 800-grit silicon carbide paper to remove cementum and create a flat surface. A schematic drawing of the sample preparation and observation methods is shown in Fig. 1. Three cavities 2 mm in diameter and 2 mm in depth were prepared in each tooth at three locations, middle (MC), and cervical (CC) thirds of the crown and the cervical third of the root (CR, Fig. 1b). The samples were prepared using a 100-µm grit regular diamond bur (149FG, Shofu, Kyoto, Japan) and a 25-µm grit with a flat end-tapered cylinder finishing bur (SF109R, Shofu) attached to a high-speed air hand piece under water coolant. All cavities were observed using SS-OCT to confirm their dimensions (Fig. 1c). Teeth specimens were separated into 2 groups (n=10 for each). For the cavities of the first group (SE group), an adhesive material (Clearfil SE Bond, Kuraray Noritake Dental, Tokyo, Japan) was applied directly, followed by a flowable composite resin injection (Estelite Flow Quick, Shade A2, Tokuyama Dental, Tokyo, Japan) in a bulk fill technique and curing for 20 s (Optilux 501, Kerr, Orange, CA, USA) with a power density of 600 mW/cm² (Fig. 1d). All materials were applied according to the instructions of the respective manufacturers. The cavities in the other group (PA group) were first pretreated by phosphoric acid using a total-etch technique (K-etchant Gel, Kuraray Noritake Dental) for 20 s, prior to performing the same steps as in the SE group preparation. After curing, all cavities were slightly finished manually with 2000-grit SiC paper to carefully remove excess resin composite and polished using a micro-engine hand piece with a silicone point. Subsequently, SS-OCT was used to assess the restorations.

**SS-OCT imaging and thermo-cycling**

The treated dental specimens were stored at 37°C in deionized water for 24 h and 2D cross-sectional images (B-scan) from 3 different angles were taken at 0, 60, and 120° planes across the cavity (Fig. 1e). The cross-section at 0° was the direction parallel to the horizontal of the plane. The specimen was marked using a pen and placed in the same orientation as previous scans. Subsequently, the teeth specimens were exposed to a thermocycling challenge (TC) for 5,000 cycles using a thermal-cycling

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**Table 1  Materials used in this study**

| Materials                        | Composition                                      | pH  | Procedure                                      |
|----------------------------------|--------------------------------------------------|-----|-----------------------------------------------|
| K-etchant Gel                    | Phosphoric acid, colloidal silica, water, and dyes.| 1.8 | Apply to cavities for 20 s, wash 15 s and dry with an air syringe for 10 s. |
| (Kuraray Noritake Dental Tokyo, Japan) |                                                     |     |                                               |
| Clearfil SE BOND                 | Primer: MDP, HEMA, hydrophilic dimethacrylate, photoinitiator, and water. | 2   | Apply the primer for 20 s, mild air blow. Apply adhesive and air blow gently. Light cure for 10 s. |
| (Kuraray Noritake Dental)        | Adhesive: MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, camphorquinone, and silanated colloidal silica. |     |                                               |
| ESTELITE FLOW QUICK              | Bis-MPEPP, TEGDMA, UDMA, silica-zirconia filler, silica-titania fillers, and CQ. | —   | Dispense in layers up to 2 mm in thickness, light cure for 20 s. |
| (Tokuyama Dental, Tokyo, Japan)  |                                                   |     |                                               |

Bis-GMA: bisphenol-A-diglycidyl methacrylate, Bis-MPEPP: bisphenol-A-polyethoxy methacrylate, CQ: camphorquinone, HEMA: 2-hydroxyethyl methacrylate, MDP: 10-methacryloyloxydecyl dihydrogen phosphate, TEGDMA: triethyleneglycol dimethacrylate, UDMA: urethane dimethacrylate.
Fig. 1  Schematic diagram of the methods used of this study. (a) Bovine teeth were polished by 800 SiC paper to obtain a flat surface to prepare the cavities. (b) Preparing 3 round cavities in incisor bovine teeth at three different locations (MC, CC, and CR). (c) Observation using SS-OCT of the cavity walls. (d) Treatment of the specimens by two-step adhesive after pre-etching (PA) or without etching (SE) and restoration with a flowable resin composite. (e) The specimens were observed with SS-OCT (3 sections of each cavity were observed). (f) Thermocycling treatment for 5,000 cycles. (g) Observation of the same three sections after TC and after 2 months of storage in water at 37°C. (h) Cutting the specimens to confirm the findings of the SS-OCT images by CLSM. MC: middle third of crown, CC: cervical third of crown, CR: cervical third of root, PA: K etchant gel+Clearfil SE Bond, SE: Clearfil SE Bond, TC: thermocycling treatment, M: months.

Fig. 2  Methods of measuring GL
GL was measured by dividing the cavity walls into 3 regions (margin, DEJ, and bottom). The separation in the DEJ between the enamel and dentin (DEJ separation) was estimated using ImageJ software for all cavities at the baseline, after TC, and after 2 months. The red arrows show the gap formation region and DEJ separation.

device (Yamato Scientific, Tokyo, Japan, Fig. 1f) to simulate aging. Cycling conditions were 5 and 55°C water baths, with dwell and transfer times of 30 and 2 s, respectively. Following thermocycling, the samples were stored at 37°C in deionized water for 2 months. Three direction SS-OCT scanning was performed immediately after thermocycling and following 2 months of storage (Fig. 1g).

Analysis of SS-OCT images
To analyze the SS-OCT images, image analysis software (ImageJ version 1.45) was used. The GL was measured from the cavity surface in the margin, DEJ, and bottom of the cavity (Fig. 2). In addition, DEJ separation between enamel and dentin were also observed in the MC and CC cavities. The raw OCT data, 2,000×1,000 pixels for each cross-section, were imported to ImageJ and a median filter size of 2 was applied to decrease background noise. Mean values were calculated for each specimen.

Confocal laser scanning microscopy (CLSM) and image analysis
Following SS-OCT observation after 2 months of storage, 6 teeth specimens from each group, for a total of 36 cavities, were randomly selected along the 0° direction from the SS-OCT images to evaluate the GL under CLSM (3D laser scanning confocal microscope, Keyence, Osaka, Japan) (Fig. 1h). The specimens were polished using silicon carbide papers to 2000 grid under
running water until reaching the same sections imaged, which were marked during observation of the samples under SS-OCT at 0°, followed by polishing with a series of 6 to 0.25 μm diamond pastes in a circular motion. The polished surface was examined under CLSM at 20× magnification. To measure the GL values in the CLSM images, each image was imported to the ImageJ program, and the GL of the restorations in each region was measured manually.

**Statistical analysis**
Statistical analyses of the results were performed using the statistical software SPSS for Windows (version 23, Chicago, IL, USA) at a significance level of α=0.05. The mean values of GL and DEJ separation were analyzed using Kruskal Wallis tests followed by Mann Whitney U tests for a pairwise comparison. The cavity region (margin, DEJ, and bottom), cavity location (MC, CC, and CR), and cavity treatment (SE and PA) were factors for the analyses. In addition, the GL values obtained both from SS-OCT and CLSM were compared using Pearson’s correlation.

**RESULTS**
GL values obtained from the SS-OCT images of the three tooth locations (MC, CC, and CR) after three observation periods (baseline, post thermal challenge, and after 2 months) are listed in Table 2. SS-OCT detected the GL changes in the enamel, dentin, and DEJ throughout the experimental period and the separation of the DEJ was indicated by bright zones with increased signal intensity (Figs. 3–5). Correlation analysis showed a significant relationship between the SS-OCT and CLSM determined GL values (R²=0.958; p<0.05; Fig. 6). The values of normality test (Shapiro-Wilk test) were less than 0.05 for all data, therefore, the non-parametric analysis was chosen. The results showed that the GL varies significantly between regions within the cavities (Table 3A) and between cavity locations (Table 3B). However, no significant difference was observed between cavity treatment methods even after 2 months (Table 3C).

![Fig. 3 Representative cross-sectional SS-OCT images and corresponding CLSM images of MC cavities in the SE group.](image)
(a) The SS-OCT image shows a good marginal sealing performance at the dentin cavity walls. (b) CLSM image of the margin of the cavity. (c) CLSM image of the bottom of the cavity. E: enamel, D: dentin, R: composite restoration, lid heads show the DEJ. The red arrow shows the gap formation at the enamel margin.

| Table 2 | The mean (μm) of the GL for both groups in different three locations and regions of teeth |
|---------|-----------------------------------------------------------------|
|         | MC  | CC  | CR  | MC  | CC  | CR  | MC  | CC  | CR  | p-value |
| SE group |     |     |     |     |     |     |     |     |     |         |
| Baseline | 5.67Ca | 25.44BcCa | 13.93Ca | 30.87Ba | 31.72Ba | 37.64Ba | 93.50Aa | 73.13Bb | ≤0.001* |
| After TC | 33.69Cc | 60.90Cc | 16.84Ca | 71.56Cb | 73.05Cb | 106.60Bc | 39.86Ca | 34.05Bb | ≤0.001* |
| After 2M | 36.26Ba | 74.03Ba | 57.29Bb | 77.85Bb | 78.40Bb | 138.37Ba | 86.12Aa | 77.85Bb | ≤0.001* |
| PA group |     |     |     |     |     |     |     |     |     |         |
| Baseline | 0.00Ca | 20.55Ca | 16.14Cb | 45.97Ab | 86.12Ab | 46.31Ab | 115.42Bc | 116.54Ca | 0.008* |
| After TC | 8.44Cb | 39.86Cb | 70.14Bc | 63.94Ab | 138.37Ab | 155.03Ab | 339.97Bb | 339.97Bb | ≤0.001* |
| After 2M | 24.79Bb | 62.00Bb | 70.26Bb | 106.22Ab | 176.70Ab | 173.00Bb | 371.31Bb | 461.03Ab | ≤0.001* |
| p-value  | ≤0.001* | ≤0.001* | ≤0.001* | ≤0.001* | ≤0.001* | ≤0.001* | ≤0.001* | ≤0.001* | ≤0.001* |

Means with the same upper-case letter indicates insignificant difference within each column. Means with the same lower-case letter indicates insignificant difference within each raw. MC: Middle third of crown, CC: Cervical third of crown, CR: Cervical third of root, SE: SE bond group, PA: Phosphoric acid etching group, TC: Thermocycling, M: Month.*: indicates that values are significant.
Cavity region
In general, the margins of the CC and CR cavities showed decreased gap formation compared to the DEJ or bottom in the SE and PA groups (Mann Whitney U-test, p<0.05). In contrast, in the MC cavity, significantly smaller gaps were observed at the baseline in the SE and PA groups and after TC in the PA group (Table 4A, p<0.05).

Cavity location
In the SE group, no significant difference in gap formation was found within the MC, CC, and CR cavity sites at the margin and DEJ (Mann Whitney U-test, p>0.05). However, a significantly longer GL was found at the CR sites compared to the MC and CC sites at the bottom of the cavity (Table 4B; Mann Whitney U-test; p<0.05).
Table 3  Significance of differences in GL among region, location and treatment

**Results of Kruskal Wallis test**

| (A)          | Region | N   | Mean Rank | p-value |
|--------------|--------|-----|-----------|---------|
| **Baseline** | Margin | 60  | 64.27     |         |
|              | Bottom | 60  | 79.68     |         |
|              | DEJ    | 40  | 106.08    | 0       |
|              | Total  | 160 |           |         |
| **After TC** | Margin | 60  | 52.26     |         |
|              | Bottom | 60  | 102.84    | 0       |
|              | DEJ    | 40  | 89.35     |         |
|              | Total  | 160 |           |         |
| **After 2M** | Margin | 60  | 56.35     |         |
|              | Bottom | 60  | 103.39    | 0       |
|              | DEJ    | 40  | 82.39     |         |
|              | Total  | 160 |           |         |

| (B)          | Location | N   | Mean Rank | p-value |
|--------------|----------|-----|-----------|---------|
| **Baseline** | MC       | 80  | 96.37     |         |
|              | CC       | 80  | 120.74    |         |
|              | CR       | 40  | 68.29     | 0       |
|              | Total    | 200 |           |         |
| **After TC** | MC       | 80  | 81.52     |         |
|              | CC       | 80  | 117.96    |         |
|              | CR       | 40  | 103.55    | 0       |
|              | Total    | 200 |           |         |
| **After 2M** | MC       | 80  | 78.62     |         |
|              | CC       | 80  | 120.33    |         |
|              | CR       | 40  | 104.6     | 0       |
|              | Total    | 200 |           |         |

| (C)          | Treatment | N   | Mean Rank | p-value |
|--------------|-----------|-----|-----------|---------|
| **Baseline** | SE        | 100 | 95.3      |         |
|              | PA        | 100 | 105.71    | 0.187   |
|              | Total     | 200 |           |         |
| **After TC** | SE        | 100 | 98.27     |         |
|              | PA        | 100 | 102.73    | 0.584   |
|              | Total     | 200 |           |         |
| **After 2M** | SE        | 100 | 100.76    |         |
|              | PA        | 100 | 100.24    | 0.949   |
|              | Total     | 200 |           |         |

N: Number of samples, MC: Middle third of crown, CC: Cervical third of crown, CR: Cervical third of root, SE: SE bond group, PA: Phosphoric acid etching group, TC: Thermocycling, M: Month.

In the PA group, no significant difference between the MC, CC, and CR sites was observed at the DEJ and bottom region (Mann Whitney $U$-test; $p>0.05$), but the GL was smaller in the MC cavity site at the margin for the baseline reading and after TC ($p<0.05$).

**Cavity treatment**

With regards the influence of cavity treatment with or without phosphoric acid pretreatment, gap formations were significantly larger in the PA group at the bottom baseline and at the margin after TC in the CR cavity (Mann Whitney $U$-test; $p<0.05$). The SE group showed larger gap formation at the margin after TC in the MC.
Table 4  Significance of differences among the cavities in various regions, locations and treatments

| (A) Region | MC | CC | CR* |
|------------|----|----|-----|
| Baseline  |    |    |     |
| Margin    | SE | PA | SE | PA | SE | PA |
| DEJ       | S  | S  | S  | S  | S  | S  |
| Bottom    |    |    |     |
| After TC  |    |    |     |
| Margin    | —  | S  | S  | S  | S  | S  |
| DEJ       | —  | —  |     |
| Bottom    |    |    |     |
| After 2M  |    |    |     |
| Margin    | —  | —  | S  | S  | S  | S  |
| DEJ       | —  | —  |     |
| Bottom    |    |    |     |

*DEJ was not included in the results of analysis

| (B) Location | Margin | DEJ* | Bottom |
|--------------|--------|------|--------|
|              | SE     | PA   |        |
| Baseline     |        |      |        |
| MC           | —      | S    | S      |
| CC           |        |      |        |
| CR           |        |      |        |
| After TC     |        |      |        |
| MC           | —      | S    | S      |
| CC           |        |      |        |
| CR           |        |      |        |
| After 2M     |        |      |        |
| MC           | —      | —    | S      |
| CC           |        |      |        |
| CR           |        |      |        |

*CR was not included in the results of analysis

| (C) Treatment | Margin | DEJ | Bottom |
|--------------|--------|-----|--------|
|              | MC     | CC  | CR    | MC | CC  | CR |
| Baseline     |        |     |       |    |     | S (PA) |
| SE           | —      | —   | —     |    |     | S (PA) |
| PA           |        |     |       |    |     |       |
| After TC     |        |     |       |    |     |       |
| SE           | S (SE) | —   | S (PA)| — | —   | — |
| PA           |        |     |       |    |     | — |
| After 2M     |        |     |       |    |     |       |
| SE           | —      | S (SE)| —   | — | —   | — |
| PA           |        |     |       |    |     | — |

MC: Middle third of crown, CC: Cervical third of crown, CR: Cervical third of root, SE: SE bond group, PA: Phosphoric acid etching group, TC: Thermocycling, M: Month. S indicates that values are significant. (–): indicates no significance. S(SE) indicates that SE group had longer gap than PA. S(PA) indicates that PA group had longer gap than SE.

cavity and after 2 months of storage in the CC cavity (Table 4C; Mann Whitney U-test; p<0.05).

DEJ separation

Most of the SS-OCT and CLSM images of the cavity walls showed enamel separation as enhanced brightness at the DEJ within the cavity (Fig. 5). The separation values of the MC and CC sites are shown in Table 5. The CC site showed significantly longer separation than the MC baseline, after TC, and after 2 months of storage in both groups (Table 5A; p<0.05). In the PA group, DEJ separation was longer than that of the SE group after 2 months of storage (Table 5B). However, within the SE group, no significant increase in DEJ separation was
Table 5 | Significance of differences in DEJ separation among location, treatment and period of time

|       | Location | N   | Mean Rank | p-value |
|-------|----------|-----|-----------|---------|
| (A)   |          |     |           |         |
| Baseline | MC      | 20  | 15.70     | 0.009   |
|        | CC      | 20  | 25.30     |         |
|        | Total   | 40  | —         |         |
| After TC | MC    | 20  | 14.35     | 0.001   |
|        | CC      | 20  | 26.65     |         |
|        | Total   | 40  | —         |         |
| After 2M | MC    | 20  | 14.90     | 0.002   |
|        | CC      | 20  | 26.10     |         |
|        | Total   | 40  | —         |         |

| (B)   | Treatment | N   | Mean Rank | p-value |
|-------|-----------|-----|-----------|---------|
| Baseline | SE      | 20  | 17.15     | 0.069   |
|        | PA       | 20  | 23.85     |         |
|        | Total    | 40  | —         |         |
| After TC | SE     | 20  | 17.00     | 0.058   |
|        | PA       | 20  | 24.00     |         |
|        | Total    | 40  | —         |         |
| After 2M | SE     | 20  | 15.78     | 0.001   |
|        | PA       | 20  | 25.23     |         |
|        | Total    | 40  | —         |         |

| (C)   | Period   | N   | Mean Rank | p-value |
|-------|----------|-----|-----------|---------|
|       |          |     |           |         |
| SE    | Baseline | 20  | 24.90     | 0.208   |
|       | After TC | 20  | 32.65     |         |
|       | After 2M | 20  | 33.95     |         |
|       | Total    | 60  | —         |         |
| PA    | Baseline | 20  | 21.10     | 0.006   |
|       | After TC | 20  | 31.85     |         |
|       | After 2M | 20  | 38.55     |         |
|       | Total    | 60  | —         |         |

N: Number of samples, MC: Middle third of crown, CC: Cervical third of crown, CR: Cervical third of root, SE: SE bond group, PA: Phosphoric acid etching group, TC: Thermocycling, M: Month.

observed after TC or after 2 months of storage compared to the baseline, while the difference was significant in the PA group (Table 5C; p<0.05).

**DISCUSSION**

Gap formation is a key factor in composite restorations failures, which is site-dependent partly due to the different structures of the teeth. To explore this factor, SS-OCT was used in this study to observe interfacial gap formation of the margin, DEJ, and bottom around the composite restorations at the MC, CC, and CR sites.

Interestingly, the gaps at the bottom of all cavities were the longest at the margin and DEJ regions (Table 2). Since polymerization shrinkage stress is considered to be an important factor in forming gaps at the bottom of cavities20), this result can be attributed to polymerization shrinkage stress and the C-factor. Previous studies have shown that a high C-factor leads to gap formation21) and in the current study, the C-factor of the cavities was approximately 3.8 (data not shown).

Comparison of the sealing performance in the SE group among the MC, CC, and CR sites revealed that the bottom of the CR exhibited increased gap formation among the three sites (Table 2). This is likely due to variance in the number and diameter of the dentinal tubules among the MC, CC, and CR sites, leading to different thicknesses of the resin-infiltrated layers that...
penetrate the dentin. A previous study reported that the densities of dentinal tubules in the root dentin is lower than that in the crown dentin. Another study showed that the dentinal tubules on the cervical region of the crown are narrower and sparser compared to typical mid-coronal regions. Achieving good bonding to dentin depends on the resin infiltration into the intratubular and intertubular dentin, which enhances bonding to the tubule walls. Therefore, large numbers of dental tubules lead to increased resin infiltration, resulting in decreased gap formation.

Comparison of sealing performance in the PA group between the MC, CC, and CR sites revealed that the GL values at the MC site were smaller than those in CC and CR sites. A previous report characterized various morphological distinctions in the enamel structure. The authors proved that prismatic and transition enamels in the CC contain randomly oriented hydroxyapatite crystals and atypical enamel prisms that are more resistant to acid dissolution compared to MC enamel. Furthermore, the enamel near the CEJ contains a large amount of carbonate substitutions, which reflects a decreased crystallinity of the hydroxyapatite and hardness of the enamel, increasing its vulnerability to gap formation. Moreover, the enamel etching treatment resulted in longer gaps at the CC site compared to the MC site. Previous studies reported the changing morphology in the post etched cervical enamel and disturbance in the prism arrangement. The resin penetration considerably decreased in the cervical etched enamel, with shorter and less abundant formed tags. Moreover, cervical etched enamel exhibited decreased bond strength and permeability in the root dentin compared to crown dentin because of the small diameters and lower numbers of the dental tubules. Accordingly, this likely reduced the hydrophilic resin infiltration capacity into the root dentin, resulting in increased gap formation.

Comparison of the SE and PA groups showed that the PA group exhibited larger gap formation than the SE group in the margin of the CR site and the bottom of the three tooth locations (Table 2). The margin of the cavities at the CR site is composed of dentin, which apparently influenced the GL in the PA group and showed longer gaps than at the MC and CC margins. Previous studies have reported incomplete infiltration of bonding with pre-etching treatment of the dentin. Furthermore, our study showed that the GL in the SE group was longer than that of the PA group at the margin of the MC and CC enamel sites. Previous reports have demonstrated that self-etching adhesive systems exhibit inferior bond strength to enamel compared to pre-etching systems. Pre-etching treatment of enamel improved the bond strengths of the self-etching adhesive. In addition, examination of the morphology of enamel surfaces revealed that application of phosphoric acid creates a deeper enamel etching pattern than the self-etch adhesive. This explains why the GL at the enamel-adhesive interface was smaller with pre-etching treatment in this study.

The key finding in this study was the enamel separation at the DEJ in the majority of the SS-OCT images. The DEJ separation at the CC site was larger than that at the MC site in both treatment groups. In addition, DEJ separation in the PA group was larger than that in SE group (Table 5). As explained above, the enamel at the CC site contains a large amount of carbonate substitutions and its hardness is decreased compared to that at the MC sites. In terms of dentin, the size and distribution of dentinal tubules at the CC site were sparse and smaller in diameter than those at the MC site. This may explain the increased separation of the DEJ at the CC site compared to that at the MC site. Previous studies have demonstrated that additional etching using phosphoric acid in enamel is recommended to improve bonding sealing performance with self-etching adhesives. However, effect of pre-etching treatment to dentin with self-etching adhesives is controversial. Although pre-etching could increase the thickness of the hybrid layer and the presence of the resin plugs, significant increase in gap formation especially at the bottom of the cavity was also reported by several studies. Our findings were in accordance with these previous studies, where phosphoric acid etching exhibited larger gap formation than self-etch adhesive in the bottom of three different tooth locations. Nevertheless, effect of phosphoric acid etching on the DEJ, which appears susceptible to the demineralization, still remains unknown. The DEJ plays a key role in protecting dentin and preventing fractures originating from the enamel from extending to the dentin. A previous study examined the mechanism of fracture transmission through the DEJ and showed that the fracture region starts in the surface enamel and when it passes the DEJ, it extends into the dentin through another surface. The structure of DEJ is rich in organic material, has less mineral content, and contains many branches of dentinal tubules. Therefore, it may contribute to the higher sensitivity to acid treatment and subsequent separation. It was reported that the DEJ region is more vulnerable to demineralization with phosphoric acid treatment, and becomes etched more deeply by phosphoric acid gel compared to enamel or dentin. This may explain our findings concerning the larger DEJ separation in the pre-etching treatment samples.

This study evaluated the GL of the cavity at the baseline, after TC, and after 2 months at the MC, CC, and CR sites using SS-OCT. Recently, composite resin materials classified as low-shrinking composites have been commercialized, which may prevent the harmful consequences of shrinkage stresses generated at the dentin-composite interface. Further experiments are needed to evaluate interfacial gap formation with various kinds of composites and to develop shrinkage-free composite restoration.

The null hypotheses of the study were rejected as (1) a significant difference in gap formation was observed for adhesive restorations in different regions of the cavity was observed, (2) a significant difference
in gap formation in various locations of the cavities was found, and (3) the SE bond showed significantly less gap formation in dentin, but a longer GL in enamel compared to acid etching treatment.

CONCLUSION

SS-OCT can be used to detect the interfacial gaps around composite restorations in a nondestructive manner. Pre-etching treatment before adhesion increases the gap formation around the composite restoration at the bottom of cavities and DEJ.

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