Micro-CT assessment of comparative radiopacity of adhesive/composite materials in a cylindrical cavity

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This study was performed to evaluate the comparative radiopacity of adhesive/resin composite materials in cylindrical cavities using micro-computed X-ray tomography (μCT). The two-step self-etch adhesive systems, Clearfil SE Bond (SE) and FL-Bond II (FL), and flowable resin composites, Beautifil Flow F10 (BF) and Clearfil Majesty ES Flow High (MJ), were used. The radiopacity of bovine tooth structures and restorative materials was measured by μCT. In addition, cylindrical cavities prepared in bovine teeth were restored with the following adhesive/composite combinations: SE-BF, SE-MJ, FL-BF, and FL-MJ. The mean gray values of the composite restorations were calculated. The threshold values of the μCT images were evaluated using the Otsu’s thresholding method. The current results show that the comparative radiopacity of the materials and tooth structure varied, which affected distinguishing the μCT images of the composite restorations in the cylindrical cavity. The proper combination of restorative materials should be considered when conducting in vitro μCT assessments of composite restorations.

Keywords: μCT, Radiopacity, Composite restoration, Bovine teeth

INTRODUCTION

X-ray computed tomography (CT) is a non-destructive imaging method in which individual projections (radiographs) are used to reconstruct a three-dimensional (3D) structure. Microfocus X-ray CT (μCT) uses a focused beam to provide higher resolution of smaller specimens in vitro2,3), which allows for high spatial resolution image recording of inner structures2,3). Nowadays, μCT is used in in vitro studies to evaluate the shrinkage of polymerized resin composites4,5), the interfacial void fraction in a resin composite material6,7), and the presence of marginal/ internal adaptation in restorations8,9).

The radiopacity of dental restorative materials, such as resin composite, ceramic, and metal alloy, should be higher than that of the same thickness aluminum filter according to the ISO standard10). Espelid et al.11) reported that the highest accuracy for radiographic diagnosis of secondary caries is obtained when the restorative material has radiopacity slightly greater than that of enamel (i.e., 2 mmeq Al). In the μCT assessment of the restorations in vitro, radiopacity contrast would improve the distinguishability of the restorations. Insufficient radiopacity of resin composite materials may make it more difficult to resolve a μCT image using software reconstruction4). In addition, there are no recommendations or guidelines (standard) proposed for radiopacity of adhesive materials, as the bonding layer might be too thin to be detected under common dental radiographs.

Although μCT imaging is gaining popularity as a research tool in dentistry, there is no in vitro study on evaluation of the radiopaque contrast of resin composite restorations by using μCT imaging. Therefore, the purpose of this study was to evaluate the comparative radiopacity of the composite restorations in a cylindrical cavity using μCT. The null hypothesis of this study was that comparative radiopacity of the materials and tooth structures did not affect discrimination of the resin composite restoration in μCT assessment.

MATERIALS AND METHODS

Materials used in this study

The adhesive and resin composites used in this study are listed in Table 1. The two-step self-etch adhesive systems Clearfil SE Bond (SE; Kuraray Noritake Dental, Tokyo, Japan) and FL-Bond II (FL; Shofu, Kyoto, Japan) and the flowable resin composites Beautifil Flow F10 (BF; shade A2, Shofu) and Clearfil Majesty ES Flow High (MJ; shade A2, Kuraray Noritake Dental) were used. Also, the aluminum wire (purity of 99.7% Al) with a diameter of 2 mm was used as a reference. The adhesives, SE and FL and the resin composites, BF and MJ were selected from several adhesive and composite materials in the pilot study. μCT analysis demonstrated that radiopacity of SE was lower than that of dentin, while radiopacity of FL was higher than that of dentin. On the other hand, the radiopacity of MJ was remarkably higher than that of BF.

Measurement of the gray values of bovine tooth structures and the adhesive/resin composite materials

The mean gray values of bovine enamel/dentin, the adhesives, the resin composites and the aluminum wire were assessed by μCT to determine the comparative
Table 1  Materials used in this study

<table>
<thead>
<tr>
<th>Code</th>
<th>Brand Name (Manufacturer)</th>
<th>Components</th>
<th>Lot No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE</td>
<td>Clearfil SE Bond (Kuraray Noritake Dental,</td>
<td>Primer: MDP, HEMA, hydrophilic dimethacrylate, water, photo-initiator, etc.</td>
<td>01746A</td>
</tr>
<tr>
<td></td>
<td>Tokyo, Japan)</td>
<td>Bond: silica filler (10.1 wt%), HEMA, MDP, Bis-GMA, hydrophilic dimethacrylate, photo-initiator, etc.</td>
<td></td>
</tr>
<tr>
<td>FL</td>
<td>FL-Bond II (Shofu, Kyoto, Japan)</td>
<td>Primer: 4-AET, methacrylic adhesive monomer, ethanol, water, etc.</td>
<td>051372</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bond: S-PRG filler (43.5 wt%), UDMA, 2-HEMA, TEGDMA, Si(OH)₄, photo-initiator, etc.</td>
<td></td>
</tr>
<tr>
<td>BF</td>
<td>Beautifil Flow F10 (Shofu)</td>
<td>S-PRG filler (40–50 wt%), glass filler, ultra-fine filler, pigment, Bis-GMA, TEGDMA, long-chain crosslinking monomer, photo-initiator, etc.</td>
<td>051323</td>
</tr>
<tr>
<td>MJ</td>
<td>Clearfil Majesty ES Flow High (Kuraray Noritake Dental)</td>
<td>silanated barium glass filler (71 wt%), TEGDMA, stabilization agent, photo-initiator, etc.</td>
<td>CR0006</td>
</tr>
</tbody>
</table>

4-AET, 4-acryloxyethyltrimellitic acid; Bis-GMA, bisphenol-A-diglycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; S-PRG, surface pre-reacted glass-ionomer; TEGDMA, triethyleneglycol dimethacrylate.

Fig. 1  Illustration of materials and tooth structure preparation for the microcomputed tomography (μCT) measurements.

Radiopacity of the materials used for composite restorations. Specimen preparation is illustrated in Fig. 1. The bovine teeth were cut using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) and cooled in water to obtain 4×4×3 mm enamel-dentin blocks. The adhesives and flowable resin composites were each added to a transparent plastic pipette tip (10 μL) and light cured for 10 s using a tungsten-halogen light-curing unit (OPTILUX 501, Kerr, Orange, CA, USA) with a light output of 600 mW/cm². The specimens were then fixed on a turntable for scanning by μCT (InspeXio SMX-100CT, Shimadzu, Kyoto, Japan). After μCT scanning, the two-dimensional (2D) images of each specimen (n=15) were obtained and analyzed using the image analysis software (ImageJ version 1.45S, U.S. National Institutes of Health, Bethesda, MD, USA). Mean gray values of the bovine teeth, restoration materials, and air were calculated. The radiopacity of air was measured outside the specimen, because no difference in mean gray value of air inside and outside the cavity was detected in a pilot study. The gray values of each material were analyzed using one-way analysis of variance and Tukey’s test. The significance level of all tests was set to α=0.05.

Specimen preparation for the cylindrically shaped resin composite restoration

Specimen preparation for the cylindrically shaped resin composite restorations is illustrated in Fig. 2. Twelve
extracted, undamaged bovine incisors were stored frozen until the experiment. The teeth were cleaned thoroughly and washed under running water to remove all adherent soft tissues. A cavity (2.0 mm diameter and 2.2 mm depth) was prepared on the labial surface of each bovine incisor using a diamond rotary cutting instrument (#202, Shofu) and ground with #800-grit silicon carbide paper under running water to standardize the cavity (2.0 mm diameter and 2.0 mm depth). The specimens were trimmed into 4×4×3 mm enamel-dentin blocks using a low-speed diamond saw (Isomet) and water as the coolant. A hole (1 mm in diameter and 0.5 mm in depth) was drilled in the side of the specimen with a diamond rotary cutting instrument (440SS ISO #010, Shofu) to set a reference landmark for the μCT scans. The following combinations of adhesives and flowable resin composites were used for the resin composite restorations: SE-BF, SE-MJ, FL-BF, and FL-MJ (n=3). The SE and FL adhesives were applied according to the manufacturers’ instructions. The MJ and BF flowable resin composites were bulk-filled and light cured for 10 s with a light-curing unit (OPTILUX501). The distance from light curing unit to the surface of the resin composite was kept at approximately 5 mm.

μCT analysis
Specimen stands were prepared for the bottom of the specimens using acrylic resin (Unifast II, GC, Tokyo, Japan). μCT scanning was performed after preparing the cavity, curing the adhesives, and filling the resin composites. The specimen was mounted on a computer-controlled turntable with synchronized rotation and axial shift. The nominal isotropic resolution of the setup was 5.0 μm. Scanning was performed as the specimen rotated 360° within an integration time of 400 s. Tube voltage was 100 kV at a current of 70 μA. The distance from the X-ray source to the detector was 300 mm. The specimen was mounted so that the X-ray beam was perpendicular to the restoration surface. The radiopacity of the specimen was converted into a gray value using the image analysis software (ImageJ version 1.45S). All 3D data were acquired as 2D images in 16-bit TIFF format with a resolution of 1,024×1,024 pixels to analyze the gray value. Image brightness was adjusted to maximal contrast obtained from the contrast between the MJ composite and air. A histogram of the gray values was prepared using the 2D image of the composite restorations.

Threshold gray values for two adjacent materials in the cavity
Using the μCT images of the resin composite restorations, the threshold gray values of the two adjacent materials in the cavity were evaluated using Otsu’s thresholding method, which is an automatic threshold selection method for image segmentation. As shown in Fig. 2, the threshold values were measured in four areas (1. air-adhesive, 2. dentin-adhesive, 3. adhesive-composite, and 4. adhesive-enamel) on the μCT images (n=3).

The Otsu’s algorithm tries to find a threshold value (t) which minimizes the weighted within-class variance. A value of t is described by the following equation:

\[
\sigma^2(t) = q_1(t)\sigma^1(t) + q_2(t)\sigma^2(t)
\]

\[
q_1(t) = \sum_{i=1}^{t} P(i) \quad \text{and} \quad q_2(t) = \sum_{i=t+1}^{N} P(i)
\]

\[
\mu_1(t) = \sum_{i=1}^{t} \frac{i \cdot P(i)}{q_1(t)} \quad \text{and} \quad \mu_2(t) = \sum_{i=t+1}^{N} \frac{i \cdot P(i)}{q_2(t)}
\]

\[
\sigma^1(t) = \sum_{i=1}^{t} \left( i - \mu_1(t) \right)^2 \frac{P(i)}{q_1(t)} \quad \text{and} \quad \sigma^2(t) = \sum_{i=t+1}^{N} \left( i - \mu_2(t) \right)^2 \frac{P(i)}{q_2(t)}
\]
It actually finds a value of $t$ which lies in between two peaks such that variances to both classes are minimum. The gray value higher than the threshold value is automatically converted to “white”, whereas a gray value lower than the threshold value is converted to “black”. Eventually, the image with gray graduations could be separately discriminated into a black/white image. However, visual discrimination of two objects is difficult, when the threshold value is close to or outside of the gray values of two objects.

**RESULTS**

*Measurement of radiopacity of the tooth structures and the adhesive/resin composite materials*

The mean gray values of air, bovine dentin, enamel, adhesive, and resin composite are summarized in Table 2. A significant difference in mean gray value was detected for each component ($p<0.05$). The mean gray value of air was the lowest among all materials, followed by that of the SE adhesive. The value of the FL adhesive was between those for dentin and enamel. The mean gray values of the resin composites (BF and MJ) were significantly higher than those of the adhesives (SE and FL) ($p<0.05$). However, the gray value for MJ was significantly higher than that of BF ($p<0.05$). The mean gray value of the aluminum wire was 39,185.0±23.7 which was located between those for dentin and FL.

**$\mu$CT images of the composite restorations**

The representative 3D-$\mu$CT images of the FL-MJ restoration are shown in Fig. 3. The 3D images taken during cavity preparation (a), when the adhesive was applied (b), and when the resin composite was cured (c) were observed.

The representative 2D-$\mu$CT images of each restoration are shown in Fig. 4. No gap was found at the marginal area of the restorations. The adhesive layers of SE and FL were approximately 70 $\mu$m thick along the cavity wall and 100–350 $\mu$m thick in the cavity corner and the cavity floor, respectively. The tooth structures and the adhesive/resin composite materials were clearly

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Mean gray values of air, tooth, adhesive, and composite used in this study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Code</strong></td>
<td>Mean gray value</td>
</tr>
<tr>
<td>Air</td>
<td>34,254.8±23.7$^a$</td>
</tr>
<tr>
<td>Bovine dentin</td>
<td>37,620.8±26.0$^b$</td>
</tr>
<tr>
<td>Bovine enamel</td>
<td>39,504.8±119.9$^c$</td>
</tr>
<tr>
<td>Adhesive</td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>35,711.1±2.8$^d$</td>
</tr>
<tr>
<td>FL</td>
<td>39,213.3±10.7$^e$</td>
</tr>
<tr>
<td>Composite</td>
<td></td>
</tr>
<tr>
<td>BF</td>
<td>39,696.9±12.3$^f$</td>
</tr>
<tr>
<td>MJ</td>
<td>44,136.1±4.1$^g$</td>
</tr>
</tbody>
</table>

Mean±SD. Number of specimen, 15. Different case letters indicate significant difference ($p<0.05$).
distinguished on the SE-BF images (Fig. 4a). However, the adhesive layer for SE was dark and close to the darkness of the air outside. The MJ composite for SE-MJ was more clearly observed than that of SE-BF (b). The adhesive/composite and enamel/composite interfaces were hard to distinguish in FL-BF (c) because of their similar dark contrast. The resin composite, adhesive, enamel, and dentin were the most visually distinguished in FL-MJ (d).

Gray values of the restorations

The histograms of the gray values for the four combination restorations on the μCT images are revealed in Fig. 5. The vertical and horizontal axes...
Table 3  Threshold value for mean gray value of two adjacent materials in the cavity

<table>
<thead>
<tr>
<th>Group</th>
<th>Code</th>
<th>Threshold value</th>
<th>Threshold value for mean gray value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE-BF</td>
<td>Air</td>
<td>35,155.8±68.3</td>
<td>34,000 37,000 40,000 43,000</td>
</tr>
<tr>
<td></td>
<td>SE-BF</td>
<td>36,735.7±206.0</td>
<td>34,000 37,000 40,000 43,000</td>
</tr>
<tr>
<td></td>
<td>SE-BF</td>
<td>37,765.0±121.8</td>
<td>34,000 37,000 40,000 43,000</td>
</tr>
<tr>
<td></td>
<td>SE-BF</td>
<td>38,395.8±384.6</td>
<td>34,000 37,000 40,000 43,000</td>
</tr>
<tr>
<td>SE-MJ</td>
<td>Air</td>
<td>35,028.8±142.2</td>
<td>34,000 37,000 40,000 43,000</td>
</tr>
<tr>
<td></td>
<td>SE-MJ</td>
<td>36,605.8±302.0</td>
<td>34,000 37,000 40,000 43,000</td>
</tr>
<tr>
<td></td>
<td>SE-MJ</td>
<td>39,884.3±347.9</td>
<td>34,000 37,000 40,000 43,000</td>
</tr>
<tr>
<td></td>
<td>SE-MJ</td>
<td>38,579.3±216.1</td>
<td>34,000 37,000 40,000 43,000</td>
</tr>
<tr>
<td>FL-BF</td>
<td>Air</td>
<td>37,168.8±172.5</td>
<td>34,000 37,000 40,000 43,000</td>
</tr>
<tr>
<td></td>
<td>Dentin</td>
<td>38,471.8±135.3</td>
<td>34,000 37,000 40,000 43,000</td>
</tr>
<tr>
<td></td>
<td>FL-BF</td>
<td>39,150.3±188.3</td>
<td>34,000 37,000 40,000 43,000</td>
</tr>
<tr>
<td></td>
<td>FL-BF</td>
<td>39,250.0±303.7</td>
<td>34,000 37,000 40,000 43,000</td>
</tr>
<tr>
<td>FL-MJ</td>
<td>Air</td>
<td>37,080.3±231.1</td>
<td>34,000 37,000 40,000 43,000</td>
</tr>
<tr>
<td></td>
<td>Dentin</td>
<td>38,395.5±319.8</td>
<td>34,000 37,000 40,000 43,000</td>
</tr>
<tr>
<td></td>
<td>FL-MJ</td>
<td>41,527.3±279.5</td>
<td>34,000 37,000 40,000 43,000</td>
</tr>
<tr>
<td></td>
<td>FL-MJ</td>
<td>39,374.7±187.5</td>
<td>34,000 37,000 40,000 43,000</td>
</tr>
</tbody>
</table>

☐: Threshold value; n=3.
*: The threshold value lower than the gray value of two materials.
indicate pixel counts and the gray values of the μCT images, respectively. The arrows on the charts indicate the mean gray value for each material (Table 2). The gray value of SE in SE-BF (Fig. 5a) was located between dentin and air, which had the lowest count of the five structures because the adhesive was very thin. The gray values for BF and enamel overlapped. The grey values of the five elements in SE-MJ were dispersed (Fig.5b). The gray values of FL, enamel, and BF in FL-BF (Fig. 5c) were close to each other. The gray values for FL and MJ (Fig. 5d) were recognized independently, except for that between enamel and FL, which were very close to each other.

Threshold of the gray value for two adjacent materials
The threshold values for two adjacent materials in the cavity are summarized in Table 3. The threshold values were located between the mean gray values of the two compared materials, except for FL-BF and FL-enamel, indicating that most of the composite restorations in this study were clearly distinguished for each structure on the μCT images. However, the threshold value of FL-BF overlapped with the gray value of FL. The threshold value of FL-enamel also overlapped with the gray values of FL and enamel (Table 3), indicating the difficulty in distinguishing the structures between FL and BF and between FL and enamel on the μCT images.

DISCUSSION
The gray value of each material was assessed using μCT to determine the comparative radiopacity of the tooth structures and the restorative materials. Because the beam-hardening effect of μCT is a drawback caused by polychromatic X-rays, a 0.2-mm thick brass filter was installed in the beam path to reduce the beam-hardening effect in this study.

Dentin contains less hydroxyapatite compared to enamel, which makes it less radiopaque than enamel (Table 2). The radiopacity of a resin composite depends on filler loading and the filler particle type. Filler particles in a resin composite with a low atomic number, such as silica and alumina, have more radiolucent characteristics, whereas filler particles with a high atomic number, such as zinc, strontium, zirconia, barium glass, barium sulfate, lanthanum, and ytterbium, have more radiopaque characteristics. The present study indicated that the radiopacity of MJ is much higher than that of BF due to different compositions and filler loading of the resin composites. The filler content of MJ was 71 wt%, containing barium glass fillers. The radiopacity of MJ was significantly higher than that of enamel (Table 2). BF contained 40–50 wt% of the S-PRG fillers, indicating radiopacity characteristics. MJ (Fig. 5c) were close to each other. The gray values of FL and MJ (Fig. 5d) were recognized independently, except for that between enamel and FL, which were very close to each other.

The SE and FL adhesives also contain filler particles in the adhesive, however, the filler loading of the adhesives was lower than those of the resin composites. SE has 10.1 wt% of silica-based micro-fillers, while the FL adhesive contained 43.5 wt% of the S-PRG fillers. The gray value of FL was higher than that of SE (Table 2). The mean gray value of FL was higher than that of dentin and close to those of enamel and BF while the mean gray value of SE was between air and dentin (Table 2). The SE adhesive is clearly discriminated from the resin composites on the μCT images (Figs. 4a and b). When evaluating leakage around a restoration with μCT, SE may be recognized as air when extracting a radiopaque structure below the dentin. In addition, the adhesive layers along the cavity wall were approximately 70 μm thick, which was difficult to detect. The FL adhesive was clearly differentiated from the MJ composite (Fig. 4d); however, it was difficult to discriminate the BF composite (Fig. 4c) because the gray values of FL, enamel, and BF closely overlapped, as shown in Fig. 5c. The FL-MJ combination was distinguishable on the μCT image (Fig. 5d).

Otsu’s thresholding method was used to analyze the visual discrimination of the two structures on the μCT images of the composite restorations. The threshold method is a process to simplify a gray image into black/white image. From the current results shown in Table 3, the threshold value was located between the two objects in this study except for the cases of FL-BF and FL-enamel. The fact demonstrated that the composite restorations in this study were almost distinguished for each other on the μCT images. However, the threshold value of FL-BF was overlapped with the gray value of FL in the FL-BF restoration. FL and BF had the same structural characteristics (Fig. 4c). Also, the threshold value of FL-enamel was overlapped with both the gray values of FL and enamel in the FL-BF and FL-MJ restorations (Table 3).

These findings indicate that the null hypothesis of this study was rejected. The low radiopacity of the adhesive materials, resulted in a hindrance to assess gap formation between the resin composite and the tooth cavity. It may therefore be difficult to detect micro-gap in the resin composite restorations of SE-MJ and SE-BF. On the other hand, it may be easy to detect the presence of gap in the FL-MJ and FL-BF groups because of the relatively high radiopaque contrast of the FL adhesive. In some previous studies, the adhesives were not used for the μCT analysis to evaluate polymerization shrinkage of the resin composite in the cavity. However, lack of the adhesive is far from the clinical situation. Zhao et al. investigated gap formation of resin composite restorations using μCT. They used ammoniacal silver nitrate solution as a tracer in the dye-penetrating method. However, it was difficult to detect the enamel gap and the gap at the closed space blocking the silver penetration, such as the gap formed at the cavity floor.

The current results emphasize that radiopacity was potentially one of the most important properties not only for composites but also for adhesive materials in terms of evaluation resin composite restorations in a clinical situation.
CONCLUSION

The comparative radiopacity of the materials and tooth structure varied, which affected distinguishing the μCT images of the restorations. The proper combination of restorative materials should be considered when conducting a μCT assessment of restorations.

CONFLICTS OF INTEREST

The authors declare no conflicts of interests.

ACKNOWLEDGMENTS

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