Micro CT Analysis on the Correlation of a Cervical Defect and the Reparative Dentin in Human Permanent Teeth

Shoichi HIGASHI, Mitsuori MAYAHARA*, Tetsuo KODAKA**, Kaoru EGAWA**, Masanori NAKMURA** and Mitsuko INOUE

Abstract: The cervical defect (CD), the so-called wedge-shaped defect in Japan, or the non-carious cervical tooth lesion of human permanent teeth may be eroded by cross tooth-brushing with and without occlusal stress, or by attachment of dental clasp for a long time. Such a CD occasionally causes dentin hypersensitivity although the reparative dentin (RD) is deposited towards the dental pulp cavity. However, the correlation of a CD and the RD in volume has not been elucidated yet except for the difference of their areas on the ground sections reported previously. In this study, we analyzed the area and volume correlations of a CD and the RD in each teeth (n=10) by micro CT analysis. The area ratio (RD/CD) was 72.4±28.6 % (r=0.856, p<0.01) on the longitudinal cut plane showing the maximum areas of a CD and the RD, which were similar to those of the ground section assumed longitudinal central line of a tooth. On the other hand, the volume ratio (RD/CD) was 18.0±8.2 % (r=0.792, p<0.01). Therefore, it is clearly illustrated by micro CT analysis that the volume ratio (RD/CD) should be extremely lower than the area ratio in the longitudinal X-ray slices and also the ground sections previously reported. As the clinical consideration, the RD formation will be alleviated dentin hypersensitivity. On the basic observations, it was accuracy illustrated that micro CT analysis was useful for the volume measurement of the RD as well as the CD.

Key words: human permanent tooth, cervical defect (CD), reparative dentin (RD), micro CT analysis, three-dimensional images.

The cervical defect (CD), the so-called wedge-shaped defect in Japan, or the non-carious cervical tooth lesion (NCTL or NCCL) at labial side of permanent teeth has been morphologically reported as tooth abfraction or abrasion. The formative process of the CD or NCTL may be induced by cross/horizontal tooth-brushing with abrasive dentifrice, by tooth-brushing under occlusal stress or bruxism, or mainly by occlusal stress for a long times. In additions, the intrinsic and extrinsic acids may cause the NCTL.

When we have happened to observe such a CD or a NCTL, some of the patients may complain dentin hypersensitivity. Base on the observation by scanning electron microscopy (SEM) no hypersensitivity will occur, if the exposed dentin on the CD surface shows almost occluded dentinal tubules under daily tooth-brushing without abrasives after the patients have stopped the tooth-brushing with abrasive dentifrices. However, the hypersensitivity may occur when most of the dentinal tubules on the CD surface are opened under the abrasive tooth-brushing. On
the other hand, it has been reported by transmitted-light microscopy with the ground sections that the reparative dentin (RD) is usually formed in the dental pulp cavity along the dentinal tubules under the CD.\(^2\)\(^-\)\(^5\) The RD may protect dentin hypersensitivity based on transmitted-light microscopic observations with the ground sections.\(^1\)\(^-\)\(^5\)

Up to date, tooth-brushing will be usually performed for the enlightenment of dental health in dentistry, whereas the cross tooth-brushing using abrasive dentifrice causes tooth enamel\(^6\)\(^-\)\(^8\) to erode as well as the dentin covered with the cementum.\(^7\)\(^,\)\(^8\)\(^,\)\(^17\)\(^,\)\(^18\) Such a daily performance may induce a CD in the labial-side cervical region of human permanent teeth. Which occasionally cause dentin hypersensitivity\(^7\)\(^,\)\(^8\) although the RD influenced by the CD formation is induced towards the dental pulp cavity.\(^2\)\(^-\)\(^5\) Moreover, the dental clasp used for dental crown attachment\(^9\)\(^,\)\(^20\) may occasionally cause the cervical region to erode as a CD. When a vital tooth is treated with the dental clasp for a long time, the friction may cause dentin hypersensitivity as well as the abfraction or abrasion by cross tooth-brushing with abrasive dentifrice,\(^7\)\(^,\)\(^8\) occlusal stress with\(^11\)\(^,\)\(^12\)\(^,\)\(^14\) and without tooth-brushing\(^9\)\(^,\)\(^13\) or acid effects.\(^15\)

On such CD or NCTL formations as mentioned above, the CD structures have been observed by the naked eye,\(^1\)\(^,\)\(^4\)\(^,\)\(^5\)\(^,\)\(^11\)\(^,\)\(^16\) transmitted-light microscopy,\(^1\)\(^-\)\(^5\) and SEM\(^4\)\(^-\)\(^5\)\(^,\)\(^10\)\(^,\)\(^15\) although the SEM studies of the three-dimensional RD structures have not been reported. Therefore, some of the morphological studies on the relationship between a CD and the RD were reported mainly by optical microscopy with the ground sections of permanent teeth.\(^2\)\(^-\)\(^5\)

From the transmitted-light microscopic observations with the longitudinal ground sections, it is known that the deposited RD is formed along the dentinal tubules under the eroded CD.\(^2\)\(^-\)\(^5\) However, the correlation between the RD and the CD was not clearly illustrated.\(^2\)\(^-\)\(^5\) As one of the reason, it is seemed to be caused by the transmitted-light microscopic judgment based on one tooth ground section in spite of the relationship between the wide cervical surface area and the narrower dental pulp cavity.

Previously, we examined by using a micro CT analyzer that the root volume measurement of rat molar was useful for an experimental research.\(^21\) Therefore, we investigated the correlation of a CD and the RD in volume in this study, and also the three-dimensional images were observed by micro CT analysis. We believe that such a micro CT analysis can show the data of a CD and the RD in volume and shape, while SEM studies could not show.\(^4\)\(^-\)\(^8\)\(^,\)\(^10\)\(^,\)\(^15\) In addition, the area ratio (RD/CD) was measured on a tooth plane showing the maximum values of a CD and the RD by micro CT analysis for the comparison with the ground sections reported previously.\(^2\)\(^-\)\(^5\) After analyzing, the ground sections were prepared and then photographed by transmitted-light microscopy for the comparison with the micro CT images.

### Materials and Methods

Recently, the collection of extracted human teeth has been very difficult because of medical ethics. In this analysis, we used permanent teeth that have remained for the histological practice course of the ground sections in Showa University School of Dentistry. From such caries-free teeth without any endodontic treatment, 10 human permanent teeth (sample number; #1 to #10) clearly showing a cervical defect (CD) in the cervical region by the naked eye were selected. Therefore, the past-records including age, sex, and other factors were unknown except for the type of teeth, which were identified as the upper incisor (1 tooth), upper canines (4 teeth), upper premolars (3 teeth), and lower canines (2 teeth). Their root tips were transversely cut with a diamond wheel for making of the standing positions in the micro CT chamber.

On the outer surface of an intact tooth before the CD formation, the assumed curve-line was established as the imagination that was connected between the intact crown-side ridge and the intact root-side ridge. On the other hand, the inner surface curve-line of
the reparative dentin (RD) deposited on the regular secondary dentin (SD) surface was decided with the connection between the both-side edges beginning the RD proliferation. In addition, the boundary line of the normal dentin and the SD has the Schreger’s line showing the regular bending of dental tubules (see Fig. 3c), whereas the dental tubules in the RD show irregular orientation and low density (see Fig. 3c). Such two-kinds of assumed curve lines were based on the longitudinal tooth slices by micro CT analysis as mentioned below.

The micro CT analysis of 10 teeth was performed with the procedures indicated in the CT analyzer (Microfocus X-Ray CT System, Inspexio SMX90-CT; Kyoto, Japan) under the conditions of the accelerating voltage of 90 kV, a specimen irradiation current of 108-μA. Figure 1, followed by our previous report, shows the reconstruction procedure by micro CT analysis. Serious longitudinal tooth sections of 44-μm at interval (Step 1) are connected with the neighboring slices (Step 2). Thus, the three-dimensional image is obtained (Step 3). The assumed intact curve-line before the CD formation and the assumed curve line between the SD and the RD (see Fig. 2a) were mildly connected in each slices by micro CT analysis.

In each teeth, the maximum area of a CD and the reparative dentin (RD) in a longitudinal cut plane calculated with the micro CT analyzer, and then an eroded CD and the deposited RD volumes were measured with a three-dimensional analyzing soft (TRI/3D; RATOC, Tokyo, Japan). Consequently, the ratios of the RD/CD area and volume were obtained. For all statistical treatments, a soft ware (Dr SPSS2, SPSS Japan Inc., Tokyo, Japan) was used. The two- and three-dimensional images were also taken using the analyzer.

After the analyses, the sample teeth were embedded in resin and then the 100- to 50-μm-thick ground sections cut throughout the assumed longitudinal central line were prepared. They were observed by transmitted-light macroscopy and transmitted-light microscopy, and then compared with the two-dimensional images by micro CT analysis.

**Results**

All the teeth used in this study more or less possessed the dental pulp cavity but not almost completely embedded with the regular secondary dentin (SD) and the irregular secondary or reparative dentin (RD). Figure 2 shows one example of the longitudinal cut slice (#6) showing the maximum areas of a CD and the RD in micro CT image out of the 10 tooth samples (#1~#10). Figure 2a is the outline image obtained from the micro CT image (Fig. 2b). The CD white area was looked like about equal to the RD white area.
Figure 3a is a higher magnification of Fig. 2b (a) and the macroscopic photograph of the ground section of a tooth (b) throughout an assumed longitudinal central line, similar to (a). The transmitted-light micrograph (Fig. 3c) shows a part of Fig. 3b. CD: cervical defect, RD: reparative dentin, C: cementum, SD: regular secondary dentin, DP: dental pulp.

Figure 4 is the histogram of the area values of a cervical defect (CD) and the reparative dentin (RD) in each 10 tooth samples (#1–#10) by micro CT analysis. Black column: eroded CD, White column: deposited RD.

Table 1  Area values of a cervical defect (CD), the reparative dentin (RD), and the RD/CD on the longitudinal cut plane showing their maximum areas based on micro CT analysis. Mean±S.D. (n=10). The difference between the area of a CD and the RD is significant (p<0.01, paired t-test).

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<thead>
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<th></th>
<th>Mean</th>
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<tr>
<td>CD area (mm²)</td>
<td>3.76</td>
<td>1.91</td>
</tr>
<tr>
<td>RD area (mm²)</td>
<td>2.67</td>
<td>1.89</td>
</tr>
<tr>
<td>RD/CD (%)</td>
<td>72.4</td>
<td>28.6</td>
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As denoted already in the materials and methods, Fig. 3c shows the transmitted-light micrograph in a part of Fig. 3b showing the boundary dotted lines among the primary dentin, the SD, the RD, and the dental pulp, respectively. The Schreger’s line showing the bend of dentinal tubules divides the primary dentin and the SD, while the RD is the area possessing irregular dentinal tubules. Such a boundary line between the SD and the RD was researched after micro CT analysis in all the 10 tooth samples.

Figure 4 is the histogram of the maximum areas of a
Fig. 5 Correlation of the area value between a cervical defect (CD) and the reparative dentin (RD) in all the 10 tooth samples ($r=0.856$, $p<0.01$, $n=10$) by micro CT analysis.

Fig. 6 Micro CT three-dimensional images of a cervical defect (CD) and the reparative dentin (RD) in one example (sample #6).

Fig. 7 Histogram of the volume values between a cervical defect (CD) and the reparative dentin (RD) in each 10 tooth samples (#1~#10) by micro CT analysis. Black column: eroded CD, White column: deposited RD.

CD and the RD in each 10 samples (#1~#10) based on the two-dimensional images by micro CT analysis. In samples #1 and #6, the RD area was somewhat wider than the CD area. However, the difference between the area of a CD and the RD is significant ($p<0.01$, paired t-test, $n=10$). The area values of the CD (3.76±1.91 mm$^2$) and the RD (2.67±1.89 mm$^2$) were shown in Table 1. The RD/CD area ratio was 72.4±28.6% and the correlation of the RD/CD was $r=0.856$ ($p<0.01$, $n=10$) (Fig. 5).

Figure 6 shows the micro CT example (#6) showing the three-dimensional image of the CD and RD volumes. Figure 7 is the histogram of an eroded CD volume and the deposited RD volume in each 10 samples based on micro CT analysis. In one example (#6), the RD volume was clearly smaller than the CD volume (Fig. 7), which differed in their areas (Fig. 4). The difference between the volume of a CD and the RD is significant ($p<0.01$, paired t-test, $n=10$). The volume values of the CD (2.56±1.57 mm$^3$) and the RD (0.44±0.38 mm$^3$) were shown in Table 2. The RD/CD ratio was 18.0±8.2 % and the correlation of the RD/CD was $r=0.792$ ($p<0.01$, $n=10$) (Fig. 8).

Table 2 Volume values of a cervical defect (CD), the reparative dentin (RD), and the RD/CD based on micro CT analysis. Mean±S.D. ($n=10$). The difference between the volume of a CD and the RD is significant ($p<0.01$, paired t-test).

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>S.D.</th>
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<tbody>
<tr>
<td>CD volume (mm$^3$)</td>
<td>2.56</td>
<td>1.57</td>
</tr>
<tr>
<td>RD volume (mm$^3$)</td>
<td>0.44</td>
<td>0.38</td>
</tr>
<tr>
<td>RD/CD (%)</td>
<td>18.0</td>
<td>8.2</td>
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Fig. 8 Correlation of the volume value between a cervical defect (CD) and the reparative dentin (RD) in all the 10 tooth samples ($r=0.792$, $p<0.01$, $n=10$) by micro CT analysis.
Discussion

Recently, as mentioned in this introduction, the tooth collection for the laboratory studies has been very difficult because of medical ethics. The 10 permanent vital teeth (#1~#10) used in this micro CT analysis were reserved for the histological practice course in Showa University School of Dentistry long ago, but the past-records including age, sex, and other factors were unknown except for the tooth class. Therefore, we inferred that the wedge-shaped defect (CD) was formed by either abrasive tooth-brushing with and without occlusal stress6~8,11,12,14) or attachment of dental clasp for a long time,10,20) but all the teeth had formed the reparative dentin (RD) on the inner surface of the regular secondary dentin (SD) adjacent to the dental pulp cavity. In additions, the odontoblast activity followed by aging was also unknown, whereas the tooth class was identified. However, the number was few or a few in each class, so we could not performed their statistical treatments. Thus, the main object in this micro CT analysis study was clearly illustrated on the correlation of the volume ratio between a CD and the RD.

Though the CD structures in the three-dimensional image were easily observed by SEM4,10) due to be eroded on the outer tooth surface, the detection of exact RD structures was difficult because the RD was formed on the SD surface towards the dental pulp cavity. The three-dimensional images of CD have been roughly divided into V and U in shape by the naked eye,1,4,5,11,15) transmitted-light microscopy with the ground sections,1~5) and SEM with the bulks.5,10) Such V- and U-shaped CD structures might be formed by abrasive tooth-brushing or attachment of dental clasps for a long time, respectively. However, the classification was difficult because their structures showed variable changes except for the typical examples.1~5,10,11,15) In the 10 sample teeth used in this study, regrettably, no typical CD shapes were detected by both naked eye and micro CT analysis.

In the two-dimensional image by micro CT analysis, the RD area was significantly wider than a CD area (p<0.01, paired t-test, n=10), but negatively in 2 samples and about equal to each other in 2 samples (see Fig. 4). The CD volume was significantly larger than a RD volume (p<0.01, paired t-test, n=10), and the RD/CD volume ratio (18.0±8.2%; r=0.792, p<0.01, n=10) was remarkably lower than the RD/CD area ratio (72.3±28.6%; r=0.856, p<0.01, n=10), which had been believed in the tooth ground sections, reported previously.2~5) In addition, the RD/CD volume ratio showed extremely lower than the RD/CD area ratio by micro CT analysis. These results will be derived from the fact that the outer surface of the intact cervical tooth forming a CD is wider area than the outer surface of the dental pulp cavity forming the SD and the RD which is followed by the arrangement of dentinal tubules succeeded seriously (SD) and un-seriously (RD) from the CD surface.2~5)

Clinical Consideration

We indicated by examine the correlation of a CD and the RD by micro CT analysis that the RD/CD volume ratio showed an extremely lower value than the area ratio. These results suggest that the RD effect might not be expected against dentin hypersensitivity for a comparison with the area ratio obtained by micro CT analysis and transmitted-light microscopy with the ground sections. Therefore, the RD formation will be never overestimated to dentin hypersensitivity. For the clinical treatments, resin cap with the tags should be necessary in the CD surface when dentin hypersensitivity is severe. If the sensitivity is slight, the patient should stop the cross tooth-brushing and perform normal tooth-brushing without abrasive dentifrices.7,8,17,18)

Conclusion Remarks

By using a micro CT analyzer, the volume or three-dimensional correlation of a CD and the RD in a tooth could be clearly illustrated, when compared with the area or two-dimensional correlation in the longitudinal cut slice of a tooth.
References

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