Anisotropy of Tensile Strengths of Bovine Dentin Regarding Dentinal Tubule Orientation and Location

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The purpose of this study was to investigate the effects of the location and orientation of dentinal tubules in the tooth on tensile strengths of the dentin. Dumbbell-shaped specimens of 12 groups from various locations and dentinal tubule orientations were prepared. The tensile test was performed in distilled water at a temperature of 37°C. The tensile strengths of the parallel to the orientation were significantly greater than those of the perpendicular to the orientation; the tensile strengths of the radicular dentin were significantly greater than those of the coronal dentin. Nevertheless, in the radicular dentin, the tensile strengths of the perpendicular to dentinal tubules differ with respect to tensile forces. These results suggest that tensile strength of the dentin varies according to the location and orientation of dentinal tubules in the tooth.

Key words: Dentin, Tensile strength, Tooth

INTRODUCTION

The mechanical properties of dentin are very important for clinical dentistry; therefore, many studies concerning the mechanical properties of dentin have been carried out. However, dentin characteristics are considered to be anisotropic regarding the location in the tooth and orientations of dentinal tubules. The density and size of the peripherical dentinal tubules are higher and larger than those of the circumpulpal dentin; the density and the size of the coronal dentin are higher and smaller than those of the radicular dentin. The mineral content of the coronal dentin is higher than that of the radicular dentin. The amount and diameter of the collagen fiber in the radicular dentin are greater and larger in comparison with those in the coronal dentin. Moreover, collagen cross-links are reported to vary with location.

Several studies have reported anisotropical mechanical properties of dentin due to dentinal tubule orientation and location, such as the three-point bending strength, diametral tensile strength, shear strength, and Vickers' hardness. Moreover, the bonding efficiencies of an adhesive resin system to dentin vary according to the dentinal tubule orientation and location. However, other studies suggest isotropical mechanical properties of dentin, such as compressive strength, shear strength, and hardness.
The tensile test is believed to be the most sensitive test method for evaluating internal structure\(^{21}\); however, information regarding the tensile properties of dentin are limited\(^{1-3,10,11,15}\). Bovine teeth were employed as a substitute for human teeth in some previous studies\(^{22}\) because the human tooth is too small to prepare tensile specimens. As a result, the relationships between the dentin tensile strength and location inside a tooth and that between the tensile strength and dentinal tubules orientation have not been clearly confirmed. Therefore, the purpose of this study was to measure the bovine dentin tensile strength in various locations in the tooth and the dentinal tubule orientation to clarify the anisotropic characteristics of dentin.

**MATERIALS AND METHODS**

**Bovine teeth**

Bovine teeth were used in this study. Central lower bovine incisors were extracted immediately after sacrificed. Teeth with a coronal or radicular fracture were eliminated. The bovine age was assumed to be from 2 to 2.5 years by dental age\(^{10}\). The bovine teeth were refrigerated within 3 hours after extraction and stored at a temperature of \(-12^\circ\text{C}\) in a freezer (221YS2, Hitachi Air Conditioning and Refrigeration, Tokyo, Japan). All teeth were utilized within 4 weeks after extraction. Before preparation, the teeth were defrosted by immersion in distilled water at room temperature for thirty minutes, and soft tissues surrounding the teeth were removed.

**Preparation of specimens**

A dentin sheet from a bovine tooth was trimmed into a dumbbell-shaped specimen for the tensile test\(^{10,11}\); one dumbbell-shaped specimen was harvested from each tooth. The twelve conditions of dumbbell-shaped specimens regarding location and dentinal tubule orientation are summarized in Table 1. Each dumbbell-shaped specimen was prepared from a rectangular block of a dentin sheet. The rectangular blocks were cut from various locations, as shown in Fig. 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dentin sheet</th>
<th>Location</th>
<th>Distance from pulp</th>
<th>Orientations to the dentinal tubules</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Type C1</td>
<td>Crown-Center</td>
<td>5.0 mm</td>
<td>Parallel</td>
</tr>
<tr>
<td>2</td>
<td>Type C1</td>
<td>Crown-Center</td>
<td>5.0 mm</td>
<td>Perpendicular</td>
</tr>
<tr>
<td>3</td>
<td>Type C1</td>
<td>Crown-Center</td>
<td>10.0 mm</td>
<td>Parallel</td>
</tr>
<tr>
<td>4</td>
<td>Type C1</td>
<td>Crown-Center</td>
<td>10.0 mm</td>
<td>Perpendicular</td>
</tr>
<tr>
<td>5</td>
<td>Type C1</td>
<td>Crown-Medial</td>
<td>5.0 mm</td>
<td>Parallel</td>
</tr>
<tr>
<td>6</td>
<td>Type C1</td>
<td>Crown-Medial</td>
<td>5.0 mm</td>
<td>Perpendicular</td>
</tr>
<tr>
<td>7</td>
<td>Type C1</td>
<td>Crown-Distal</td>
<td>5.0 mm</td>
<td>Parallel</td>
</tr>
<tr>
<td>8</td>
<td>Type C1</td>
<td>Crown-Distal</td>
<td>5.0 mm</td>
<td>Perpendicular</td>
</tr>
<tr>
<td>9</td>
<td>Type C2</td>
<td>Crown-Distal</td>
<td>5.0 mm</td>
<td>Perpendicular</td>
</tr>
<tr>
<td>10</td>
<td>Type R1</td>
<td>Root</td>
<td>—</td>
<td>Perpendicular</td>
</tr>
<tr>
<td>11</td>
<td>Type R2</td>
<td>Root</td>
<td>—</td>
<td>Perpendicular</td>
</tr>
<tr>
<td>12</td>
<td>Type R1</td>
<td>Root</td>
<td>—</td>
<td>Perpendicular</td>
</tr>
</tbody>
</table>
Fig. 1 Location of rectangular block groups in the dentin sheet.

Two dentin sheets (C1, R1) were prepared by medio-distal section; the other two dentin sheets (C2, R2) were prepared by labio-lingual section. Locations of twelve rectangular blocks in each dentin sheet are indicated.

The bovine teeth were sectioned at the cement-enamel junction. Four types of 1.0 mm thickness dentin sheets were prepared. The 1.0 mm-thickness dentin sheet of Types C1 and C2 from the coronal portion of the teeth were prepared by medio-distal and labio-lingual sectioning, respectively. The dentin sheets of Types R1 and R2 from the radicular portion were obtained by medio-distal and labio-lingual section, respectively. One coronal sheet, C1, was sectioned along the plane containing the incisal edge of the crown, and the lingual edge of the pulpal chamber appeared in the radicular surfaces and along a 1.0 mm labio-lingual plane to the first section. The other coronal sheet, C2, was sectioned along the plane containing the distal incisal edge of the pulpal chamber and along a 1 mm medial parallel to the first section. The R1 radicular dentin sheet was cut along the plane containing the incisal edge of the crown and the center edge of the apex, and along a 1 mm labio-lingual plane to the first section; the other radicular sheet, R2, was labio-lingually sectioned along the plane at a right angle of the R1 sheet which included the center portion.

Twelve groups, Groups 1-12, of rectangular blocks (8.0x3.0x1.0 mm) were prepared according to the following procedure. The central portion of the Group 1 block from the C1 dentin sheet was located in the central area, 5 mm from the labial pulpal edge in the dentin sheet; the long axis of the block was parallel to the dentinal tubule orientation. The central portion of Group 2 was identical to that of Group 1, but the long axis of the block was perpendicular to the dentinal tubule orientation. The central portion of the Group 3 block from the C1 dentin sheet was located in the central area, 10 mm from the labial pulpal edge; the long axis of the block was parallel to the dentinal tubule orientation. The central narrow portion of Group 4 was identical to that of Group 3, but the long axis of the specimen was
perpendicular to the dentinal tubule orientation. The central portion of the Group 5 block prepared from the C1 dentin sheet was located in the medial area, 5 mm from the pulpal edge; the long axis of the specimen was parallel to the dentinal tubule orientation. The central portion of Group 6 was identical to that of Group 5, but the long axis of the specimen was perpendicular to the dentinal tubule orientation. The central portion of the Group 7 block prepared from the C1 dentin sheet was located in the distal area, 5 mm from the pulpal edge; the long axis of the specimen was parallel to the dentinal tubule orientation. The central portion of Group 8 was identical to Group 7, but the long axis of the specimen was perpendicular to the dentinal tubule orientation. The central portion of Group 9 block from the C2 dentin sheet was located in the center area, 5 mm from the labial pulpal edge; the long axis of the block was perpendicular to the dentinal tubule orientation. The central portion of Group 9 was identical to Group 7 and Group 8, but the long axis of the block was perpendicular to the dentinal tubule orientation. The central portion of the Group 10 block from the R1 dentin sheet was located in the area near the cervical dentin, 1.5 mm from the pulpal wall; the long axis of the block was perpendicular to the dentinal tubule orientation. The central portion of the Group 11 block from the R2 dentin sheet was located in the area near the cervical dentin, 1.5 mm from the pulpal wall and the long axis of the specimen was perpendicular to the dentinal tubule orientation. The central portion of Group 10 was identical to that of Group 11. The central portion of the Group 12 block from the R1 dentin sheet was located in the area near the root apex dentin, 1.5 mm from the pulpal wall; the long axis of the block was perpendicular to the dentinal tubule orientation.

The dumbbell-shaped specimens with a 1.0×1.0×1.5 mm central narrow portion were prepared from the rectangular block using a profile machine consisting of a water spraying air-turbine handpiece (DA 231, Takara Belmont, Osaka, Japan), a specimen stage and the driving unit of the stage, following previous reports. The specimen was motor-driven and ground with a superfine diamond bar (SF114S, Shofu, Kyoto, Japan) attached to the air-turbine according to the movement of the guide roller along the dumbbell-shaped template. All surfaces of the central narrow portion were finished with 1200 grit wet silicon carbide paper.

**Tensile test procedure**

The tensile strengths of the dentin specimens were determined according to previous reports. Geometrics of the central portion of the dumbbell-shaped specimen were measured using a micrometer with a 0.001 mm precision (MD210-25, Mitsutoyo, Tokyo, Japan). Dentin specimens were mounted on a universal test machine (Type 1123, Instron, Canton, MA, USA) using jigs for the tensile test. The distance between the upper and lower jigs holding the dentin specimen was approximately 4 mm. The tensile test was performed in distilled water at a temperature of 37±0.5°C at a crosshead speed of 0.5 mm/min. The tensile strengths were calculated from the fracture load divided by the original central cross-sectional area. Ten specimens were employed for each test group. The tensile strengths were statistically analyzed by
the one-way ANOVA and Tukey's multiple comparison tests. A statistical software program (JMP3.22, SAS, Cary, NC, USA) was employed at a significant level of 5%.

Scanning electron microscope (SEM) observation
The surfaces of the specimens after the tensile test were coated with gold using an ion spattering machine (Ion Coater IB-3, Eiko Engineering, Tokyo, Japan) and observed using a scanning electron microscope (S-4500, HITACHI, Tokyo, Japan). The dentinal tubule orientation was confirmed and fracture origins were estimated from the gradation of surface roughness and the running direction of the crack propagations.

RESULTS

Tensile strength
All specimens were fractured at the central narrow portions. The tensile strengths are summarized in Table 2. The tensile strengths were significantly different among the groups.

Regarding the tensile strengths from the coronal dentin, the tensile strengths parallel to the tubules, 77.6 to 79.6 MPa (Groups 1, 3, 5 and 7), were significantly greater than those perpendicular to the tubules, 34.9 to 44.5 MPa (Groups 2, 4, 6 and 8). However, the effect of coronal location (center, medial, distal) was not significant when tubule orientation was identical. With respect to the difference between coronal and radicular dentin perpendicular to the dentinal tubules, the strengths from the radicular dentin (Groups 10 and 12) were significantly greater than those from the coronal dentin (Groups 2, 4, 6 and 8).

Comparing Groups 7, 8 and 9 whose central portions were of similar coronal location, the tensile strength of Group 7 was significantly greater than those of

| Table 2 Means and standard deviations of tensile strength of each group |
|----------------------|----------------------|
| Group    | Tensile strength (MPa) |
| 10       | 90.1±7.19          |
| 12       | 81.0±8.27          |
| 3        | 79.6±7.56          |
| 5        | 78.8±4.97          |
| 7        | 77.6±7.45          |
| 1        | 76.7±6.12          |
| 9        | 56.3±8.84          |
| 8        | 44.5±5.13          |
| 11       | 43.3±3.11          |
| 4        | 43.2±7.04          |
| 6        | 35.9±6.83          |
| 2        | 34.9±4.37          |

n=10, mean±S.D.
Groups connected by a horizontal line are not significantly different according to Tukey's multiple comparison test (p<0.05).
Fig. 2 Typical fracture surface after tensile test.
a: Group 1 at low magnification, b: Group 1 at high magnification, c: Group 2 at
low magnification, d: Group 2 at high magnification, e: Group 10 at low magnification,
f: Group 10 at high magnification, g: Group 11 at low magnification, h: Group
11 at high magnification
Groups 8 and 9. However, the tensile strengths of Groups 8 and 9 were not significantly different; the tensile stress direction of Groups 8 and 9 was perpendicular to the tubules. In contrast, the tensile strengths of the radicular dentin, Group 10 and Group 11, were significantly different in spite of a similar location and dentinal tubule orientation.

**SEM observation**

Typical fracture surfaces after the tensile test are shown in Figs. 2a-h. The relationships between crack paths and dentinal tubule orientation did not always coincide. Fracture origins could be identified from approximately 80% of the fractured specimens.

Giant tubules were observed in half of the fractured surfaces. One or two specimens in each group were estimated to be fractured at these giant tubules; however, not all giant tubules acted as the fracture origin; therefore, the existence of giant tubules was not an important criteria for determination of fracture origin.

The fracture surface of coronal specimens stressed parallel to dentinal tubule orientation is shown in Fig. 2a. Nearly all fracture origins were estimated to be at the periphery or edge of the specimen. Dentinal tubules were cross-sectioned at a high magnification (Fig. 2b). These findings were almost equal regarding Groups 1, 3, 5 and 7. Fig. 2c shows a coronal fracture surface which was stressed perpendicular to dentinal tubule orientation. Half of the estimated fracture origins were located inside the specimen. Dentinal tubules were longitudinally sectioned at a high magnification (Fig. 2d), and the intertubular and peritubular dentin were not clearly classified. These findings were almost equal regarding Groups 2, 4, 6, 8 and 9.

The fracture surfaces of radicular specimens (Fig. 2e) were more complicated than those of coronal specimens. Half of the fracture origins were estimated to be located at the periphery of the specimen. Longitudinally sectioned dentinal tubules were observed at a high magnification (Fig. 2f). Sizes and density of dentinal tubules in the root were smaller and less than those in the crown, respectively. Fractured surfaces of radicular intertubular dentin were rough compared to those of the crown. These findings were almost equal with respect to Groups 10 and 12. Fig. 2g shows the fracture surface of Group 11; half of the fracture origins were estimated to be located at the periphery of the specimen. Longitudinally-sectioned dentinal tubules were observed at a high magnification (Fig. 2h). The fractured intertubular dentinal surface showed a rough pattern. The size and density of dentinal tubules were almost equal to those of Groups 10 and 12.

**DISCUSSION**

In this study, bovine teeth of identical dental age were employed to decrease the deviation of the mechanical properties of each tooth. During tensile specimen preparation and the tensile test, the specimen was kept wet to avoid dyhydration, since dyhydration of the dentin increases tensile strength.
Fig. 3 Schematic cross-sectioned net area. If the dentinal tubules were round and straight, arranged in a regular triangle, the distance to the center of tubule was 8.21 μm. Therefore, the cross-sectioned net area of an apparent perpendicular to the dentinal tubule orientation 1 mm² specimen was calculated as 0.89 mm², whereas that parallel to the dentinal tubule orientation varied from 0.65 to 1.00 mm².

Tensile strengths in this study ranged from 34.8 MPa to 90.1 MPa, which is similar to previously reported values, 36.7 MPa to 128.6 MPa15–21. In our data, the tensile strengths parallel to the dentinal tubules were greater than those perpendicular to the dentinal tubules, and the tensile strengths of the radicular dentin were greater than those of the coronal dentin. These results were consistent with Sano’s report8). However, this contrasts with a report by Lertchirakan et al.21) in which the tensile strengths of human coronal dentin parallel and perpendicular to dentinal tubules were 36.7 MPa and 60.3 MPa, respectively, and those of human radicular dentin parallel and perpendicular to dentinal tubules were 41.1 MPa and 59.6 MPa, respectively.

The effects of dentinal tubule orientation on the dentin tensile strengths were considered as morphological and structural factors. Regarding morphological factors, one factor is the discrepancy between the cross-sectioned net area parallel to the dentinal tubules and that perpendicular to the dentinal tubules; the other is the cross-sectioned shapes of the dentinal tubules. The cross-sectioned net area parallel to dentinal tubules and perpendicular to dentinal tubules is illustrated in Fig. 3. The number and diameter of bovine coronal dentinal tubules are reported as 17130/mm² and 2.85 μm, respectively19. When the shape of the coronal dentinal tubules was considered as a round and straight tube and these tubes were arranged in a regular triangle, the distance to the center of each dentinal tubule was calculated as 8.21 μm.
Therefore, the cross-sectioned net area of an apparent 1 mm² specimen perpendicular to the dentinal tubule orientation was calculated as 0.89 mm². The net area of an apparent 1 mm² specimen parallel to the dentinal tubule orientation varied from 0.65 to 1.00 mm², depending on the cross-sectioned plane. A tensile fracture usually occurs at the minimum cross-sectioned plane. Therefore, the tensile strength parallel to the dentinal tubule orientation was greater than that perpendicular to the dentinal tubule orientation. Moreover, the ideal shape of the cross-sectioned dentinal tubules parallel and perpendicular to this orientation was round and rectangular, respectively. These cross-sectioned dentinal tubules were considered as potential precracks. The sizes of these precracks parallel to the dentinal tubule orientation were much greater than those perpendicular to the dentinal tubule orientation; therefore, the tensile strength stressed perpendicular to the dentinal tubules might be greater than that parallel to the dentinal tubules. This finding suggested that the estimated fracture origin distribution was related to the applied tensile stress direction; half of the estimated fracture origins of Groups 1, 3, 5 and 7, stressed parallel to the dentinal tubules, were located at the periphery or edge of the specimen, while half of the estimated fracture origins of Groups 2, 4, 6, 8 and 9, stressed perpendicular to the dentinal tubules, were located inside the specimen.

Dentin is a complex structure of hydroxyapatite and collagen. It has been reported that the strength of hydroxyapatite has little effect on the anisotropic mechanical properties of bone. The structural factor of dentin on the mechanical properties is considered mostly to be the orientation of collagen fiber. Collagen in the dentin exists as a bundle of fibers. The main direction of these collagen fibers, known as fibers of the dentinal matrix, is usually parallel to the growth line; these fibers of the dentinal matrix can be identified by etching the surface using, for example, 5% formic acid solution immersion. Fig. 4 shows the typical etched fractured surface of the coronal dentin of Group 9. The orientation of fibers of the dentinal matrix observed in the fracture surface after etching of Group 9. White arrows indicate observed fibers of dentinal matrix.
dentinal matrix was observed oblique to the dentinal tubules. These findings were almost the same among Groups 7, 8 and 9. Therefore, the structural factor of tensile strength of coronal dentin was not so significant regarding Groups 7, 8 and 9. The fibers of dentinal matrix orientation of bovine and human dentin have not been clearly confirmed. Lertchirakan et al. employed human premolars, but did not investigate fibers of dentinal matrix orientation. These fibers might be the reason for discrepancies between our results and their previously reported human dentin results.

With respect to the radicular dentin, fibers of the dentinal matrix are reported mainly along the tooth axial direction. This finding provided a suitable explanation concerning the difference in tensile strengths between Groups 10 and 11. The tensile stress in Group 10 coincided with the fibers of dentinal matrix orientation. However, that in Group 11 was perpendicular to the fibers of the dentinal matrix. Moreover, the shape of the dentinal tubules in the radicular dentin was usually oval-shaped with a long axis identical to the tooth axis. Therefore, the cross-sectioned diameter of the dentinal tubules in Group 11 was greater than that in Group 10. As a result, the tensile strength of Group 11 was less than that of Group 10.

The orientation of fibers of the dentinal matrix in Groups 10 and 12 was identical to the applied tensile stress; therefore, the tensile strengths of Groups 2, 4, 6, 8 and 9 might be significantly less than Groups 10 and 12 because of a mismatch between tensile stress and fibers of the dentinal matrix in spite of the similar dentinal tubule orientation. Moreover, tensile strengths of the radicular dentin were twice as great as those of the coronal dentin. The number and sizes of dentinal tubules in the coronal dentin are reported to be smaller and more dense than those in the radicular dentin, respectively, and the radicular dentinal tubules are more irregular and more complicated in branching. Additionally, the amount of collagen in the root is reportedly greater than in the crown; the area percentage of collagen fibers in the coronal dentin is 71.2%, while that in the radicular dentin is 60%. All these factors might be significant to show the difference of tensile strength between the coronal dentin and the radicular dentin.

CONCLUSION

The tensile strength of bovine dentin in various locations and dentinal tubule orientation were determined using dumbbell-shaped specimens. The tensile strengths of the coronal dentin parallel to the dentinal tubule orientation, 76.7 to 79.6 MPa, were significantly greater than those perpendicular to the dentinal tubule orientation, 34.9 to 44.5 MPa. The tensile strengths of the radicular dentin parallel to the dentinal tubule orientation and the tooth axis, 56.3 to 90.1 MPa, were significantly greater than those perpendicular to the tooth axis, 43.3 MPa. These results suggest that the tensile strength of dentin differs depending on the dentinal tubule orientation and location.
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