Relation between incremental lines and tensile strength of coronal dentin

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INTRODUCTION

Dentin is the most abundant mineralized tissue in the tooth. It constitutes the main bulk of a human tooth by both weight and volume; thus dentin plays a critical role in maintaining the structural integrity of teeth. A good knowledge of the mechanical properties of dentin is understandably important to predicting the effects of loading, stress, and fatigue on teeth.

Dentin is a hydrated biological composite which comprises inorganic hydroxyapatite crystallites (~5 nm thick, 70% by weight), organic type-I collagen (~50–100 nm diameter, 18% by weight), and water (12% by weight). Dentinal tubules run continuously from the dentin-enamel junction (DEJ) to the pulp in the crown, and from the cementum-dentin junction (CEJ) to the pulp canal in the root 11. Tubule lumina are surrounded by a collar of hypermineralized peritubular dentin, and the substance between dentinal tubules is known as intertubular dentin.

The regular, almost uniaxial, alignment of dentinal tubules thus causes the mechanical properties of dentin to be orientation-dependent. To date, many studies have investigated the anisotropic mechanical properties of dentin as a function of dentinal tubule orientation 2-14. An assumption made in many of these studies was that dentinal tubules ran perpendicular to incremental lines. This assumption was not valid in many cases.

During dentinogenesis or dentin formation, mineralization can progress in a globular (spherical) or linear pattern. Incremental lines mark the normal linear pattern of dentin deposition and have been recognized histologically in the dentin of vertebrates, as documented by Owen 15, Andresen 16, and von Ebner 17. The banding patterns of incremental lines show the growth pattern of dentin. Where accentuated incremental lines are noticed, it was because of disturbances in the mineralization process during dentin formation 18.

Dentinal tubules are surrounded by mineralized collagen fibrils, and incremental lines are oriented parallel to the collagen fibrils. In a study by Currey et al., they found a strong dependence of the elastic properties of narwhal tusk dentin on the orientation of mineralized collagen fibrils 19. Accordingly then, incremental lines in dentin might hold the key to better understanding the mechanical properties of dentin, particularly in terms of tensile strength. However, although toughening mechanisms based on the presence of collagen fibrils have long been proposed for mineralized biological tissues like bone and dentin, no direct evidence for their precise role has ever been provided 20. Furthermore, the angle between incremental lines and dentinal tubules is not fixed. Given the inconclusive evidence about the effects of the orientation of collagen fibrils on the mechanical properties of dentin, and now coupled with an inconclusive orientation of incremental lines to dentinal tubules, there raised a question on the dubious influence of incremental lines on the tensile strength of dentin.

Morphological and compositional differences exist among different tooth types and species, which then lead to different mechanical behaviors in different teeth 13. For incremental lines, their banding patterns vary with tooth type 22. The patterns of coronal incremental lines in incisors differ from those in molars, and it was suggested to be due to morphological differences between these two tooth types. Few studies have evaluated the influence of incremental lines, of different locations and orientations, on the mechanical properties of coronal dentin. The aim of this study was to evaluate and compare the tensile...
strengths of coronal dentin of human molars and bovine incisors with respect to incremental lines with different tensile orientations (parallel versus perpendicular). The hypothesis of this study was that the tensile strength of coronal dentin would not differ as a function of the orientation of incremental lines to tensile loading.

MATERIALS AND METHODS

Human and bovine teeth

Extracted, caries-free, human third molars were obtained for this study from patients of 28–46 years old under informed consent. Bovine lower central incisors were extracted immediately after sacrifice from animals assumed to be 2–2.5 years old according to their dental age. Immediately after extraction, teeth were stored in Hank’s balanced salt solution (HBSS) at 4°C until use. Teeth were used within 1 month of their extraction.

The protocol of this study was approved by the Ethics Committee of Showa University School of Dentistry.

Specimen preparation

1. Human molar coronal dentin specimens

Ten human third molars (5 maxillary and 5 mandibular, 4 male and 6 female) were used in this study. Using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) under copious water cooling, the human molars were sectioned longitudinally along the mesiodistal plane in a direction parallel to the long axis of the tooth (Fig. 1a) and approximately midway between the buccal and lingual surfaces (Fig. 1b). Two dentin slabs, each approximately 1-mm thick, were sectioned from each tooth.

Rectangular blocks (3.0×3.0×1.0 mm) were prepared from each dentin slab in the coronal area (Figs. 1c, d) for experimental groups HPa and HPe (Table 1). Specimens in HPa were subjected to tensile loading acting parallel to the incremental lines (arrow orientation, Fig. 1c), whereas HPe specimens were subjected to tensile loading acting perpendicular to the incremental lines (arrow orientation, Fig. 1d). The dimensions of the central portion of HPa specimens were identical to those of HPe specimens.

Incremental lines are shown in Fig. 1e. Dentinal tubules were nearly perpendicular to the incremental lines (Fig. 1f). Therefore, the angles between tensile orientation and dentinal tubules were 90° for HPa specimens and 0° for HPe specimens.

2. Bovine incisor coronal dentin specimens

Twenty bovine lower central incisors were used in this study and divided randomly into two experimental groups, BPa and BPe (Table 1). Using the low-speed diamond saw, 1-mm-thick dentin slabs were prepared from the labial surfaces of bovine incisors. As per the preparation procedure in previous reports for BPa group, each dentin slab was cut parallel to the plane which contained the incisal edge and at the nearest point to the root apex on the cervical line. Each dentin slab was cut at 2.4–3.4 mm from the labial surface and it ran parallel to the incremental lines (Fig. 2a). In BPe group, the bovine teeth were sectioned longitudinally along the buccolingual plane in a direction parallel to the long axis of the tooth, approximately midway between the mesial and distal surfaces (Fig. 2b). The orientation between incremental lines and dentinal tubules is shown in Fig. 2c.

Rectangular blocks (3.0×3.0×1.0 mm) were prepared from each bovine incisor crown dentin slab for experimental groups BPa and BPe (Table 1). Specimens in BPa were subjected to tensile loading acting parallel to the incremental lines (arrow orientation, Fig. 2d), whereas BPe specimens were subjected to tensile loading acting perpendicular to the incremental lines (arrow orientation, Fig. 2e). The dimensions of the central portion of BPa specimens were identical to those of BPe.

The dentinal tubules were oblique to the incremental lines (Figs. 2c–2e). Therefore, the angles between tensile orientation and dentinal tubules were about 35° (BPa) and 55° (BPe).

3. Dumbbell-shaped tensile test specimens

To obtain dumbbell-shaped specimens from rectangular blocks of human molar or bovine incisor dentin, a superfine diamond cutting bar (211SF, Shofu, Kyoto, Japan) and an air-turbine hand piece (DA 231, Takara Belmont, Osaka, Japan) attached to a profiling machine were used (Fig. 3a). As specimen was moved by a motor, the roller guide followed the template. The turbine head, which moved up and down, slid along the template and produced uniformly shaped specimens.

Dumbbell-shaped specimens with a narrow central portion (1.0×1.0×1.5 mm) (Fig. 3b) were thus produced. Surfaces of the narrow central portion were finished with 1200-grit wet silicon carbide papers.

Table 1 Experimental groups of this study according to tooth type and tensile orientation

<table>
<thead>
<tr>
<th>Group</th>
<th>Tooth type</th>
<th>Tensile orientation to incremental lines</th>
<th>Angle between tensile orientation and dentinal tubules</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPa</td>
<td>Molar</td>
<td>Parallel</td>
<td>90°</td>
</tr>
<tr>
<td>HPe</td>
<td>Molar</td>
<td>Perpendicular</td>
<td>0°</td>
</tr>
<tr>
<td>BPa</td>
<td>Incisor</td>
<td>Parallel</td>
<td>35°</td>
</tr>
<tr>
<td>BPe</td>
<td>Incisor</td>
<td>Perpendicular</td>
<td>55°</td>
</tr>
</tbody>
</table>
Fig. 1  Schematic representation of the location of dentin slab in human molar tooth. (a) Side view of tooth with longitudinal sectioning along mesiodistal plane in a direction parallel to long axis of tooth; (b) Top view of tooth which shows sectioning midway between buccal and lingual surfaces. Location of tensile test specimens in human molar coronal dentin for (c) HPa; and (d) HPe, where arrows indicate tensile orientations. Microscopic views of dentin specimens: (e) Incremental lines (white lines) in coronal dentin; (f) Perpendicular orientation between incremental lines and dentinal tubules.
**Tensile strength test**

Ten specimens of each experimental group were employed for tensile testing. Tensile tests were performed in HBSS at a temperature of 37±0.5°C (Fig. 3c), where dumbbell-shaped dentin specimens were mounted on a universal test machine (TA-XT2, Eko Co., Tokyo, Japan) using jigs (Fig. 3d). Crosshead speed was 0.5 mm/min. Mean tensile strength of the specimens was calculated.
for each group, and the results were analyzed using two-way ANOVA and Tukey’s multiple comparisons test. Statistical significance was preset at $\alpha=0.05$.

**Scanning electron microscopy (SEM)**

After tensile strength testing, specimens were fixed in 10% neutral buffered formalin for at least 8 h, rinsed with de-ionized water for 1 min, dehydrated in ascending grades of ethanol, immersed in hexamethyldisilazane (HMDS) for 10 min, and air-dried.$^{27}$ Fractured and side surfaces of the specimens were sputter-coated using an ion sputtering machine for 5 min (E-1030 Ion Sputter Coater, Hitachi, Tokyo, Japan) and then examined by SEM (S-4700, Hitachi, Tokyo, Japan). Acceleration voltage was 10 kV, and working distance was 12 mm.

**RESULTS**

**Tensile strengths of human molar dentin and bovine incisor dentin**

Figure 4 shows the mean tensile strengths of the four experimental groups of this study. Mean tensile strengths of HPa and HPe were $58.6 \pm 6.8$ MPa and $35.6 \pm 2.9$ MPa respectively. Mean tensile strengths of BPa and BPe were $62.3 \pm 5.7$ MPa and $37.8 \pm 4.2$ MPa respectively. BPa had the highest tensile strength, while HPe had the lowest. Two-way ANOVA revealed significant differences in tensile strength ($p<0.05$) between parallel groups (HPa and BPa) and perpendicular groups (HPe and BPe).

**SEM observations**

Typical fractured surfaces of HPa, HPe, BPa, and BPe specimens after tensile strength testing are shown in Figs. 5a–d respectively. Dentinal tubules surrounded by
peritubular and intertubular dentin were observed on the fractured surfaces, with distinct boundary between peritubular dentin and intertubular dentin. These SEM images also confirmed that tensile forces to the dentinal tubules were perpendicular (HPa), parallel (HPe), and oblique (BPa and BPe).

**DISCUSSION**

**Effect of specimen size and shape on tensile strength in dentin**

In our previous study\(^{24}\), when the direction of loading was directed parallel to the incremental lines in bovine coronal dentin (i.e., BPa), tensile strength was measured as 78.1±6.1 MPa. In that study\(^{24}\), dumbbell-shaped specimens with a narrow central portion (1.0×1.0×1.5 mm) trimmed from rectangular blocks (8.0×3.0×1.0 mm) were used. In the present study, tensile strength of BPa was measured lower at 62.3±5.7 MPa.

It has been reported that specimen size and shape affected tensile strength\(^{28-31}\). However, it was not possible to perform tensile testing for BPe, HPa, HPe groups using the specimen size of previous study\(^{24}\). If strict adherence to previous specimen size\(^{24}\) were to be observed in this study, it would mean that investigations for different tensile orientations would be forfeited.

To overcome the problem associated with specimen size, we employed miniaturized dumbbell-shaped specimens for tensile testing. For incisors, bovine central incisors were used because of a larger crown than the human incisors, hence overcoming the size limitations.

**Fig. 4** Mean tensile strength of each experimental group, where * indicates presence of statistical difference between groups (\(p<0.05\)).

**Fig. 5** Fractured surfaces after tensile testing: (a) HPa; (b) HPe; (c) BPa; and (d) BPe.
posed by human incisors. *In vitro* tensile strength tests were carried out in this study using dumbbell-shaped specimens in Hank’s balanced salt solution at body temperature, simulating *in vivo* oral cavity conditions as well as maintaining the structural integrity of teeth.²⁰

**Importance of dumbbell-shaped specimens for tensile testing**

Using strain concentration tests and impression-induced damage tests, Wang¹³ had shown that in coronal dentin, a circular crack formed and enveloped the radial cracks during loading. This indicated that coronal dentin is isotropic. However, the tensile properties and fracture behavior of coronal dentin showed anisotropy as a function of the orientation of incremental lines.

Specimen size and shape determine tensile test results, and dumbbell-shaped specimens were reportedly useful for identifying defects easily.⁵,¹⁹,²³⁻²⁶. For this reason, a profiling machine was used to produce uniformly shaped dumbbell specimens in this study, and that the surfaces of the narrow central portion were finished with 1200-grit wet silicon carbide papers.⁵,¹⁹,²³⁻²⁶. Results of this study revealed that the mechanical properties of coronal dentin displayed pronounced anisotropy as a function of the orientation of incremental lines. Therefore, the null hypothesis of this study was rejected because the tensile strength of coronal dentin differed as a function of the orientation of incremental lines.

**Influence of tooth type on structural anisotropy**

In dentin, incremental lines are oriented parallel to the collagen fibrils, which surround the dentinal tubules. In the dumbbell-shaped specimens used in this study, the long axis of narrow central portion corresponded to the tensile loading direction. In other words, tensile loading orientations to the incremental lines in this study also applied to the collagen fibrils.

In the present study, loading was applied in both parallel and perpendicular directions to the incremental lines in both human molars and bovine incisors. Results showed that when tensile loading was applied parallel to the incremental lines, and hence the collagen fibrils, higher tensile strengths were yielded than when applied perpendicularly. Further, the tensile strengths of HPa (human molar dentin with tensile orientation parallel to the incremental lines) and BPa (bovine incisor dentin with tensile orientation parallel to the incremental lines) were not significantly different (p>0.05), and the tensile strengths of HPe (human molar dentin with tensile orientation perpendicular to the incremental lines) and BPe (bovine incisor dentin with tensile orientation perpendicular to the incremental lines) were also not significantly different (p>0.05).

When under the same tensile loading orientation to the collagen fibrils (i.e., parallel or perpendicular), there were no statistically significant differences in tensile strength between the two tooth species. These results showed that collagen fibrils in two different types of teeth, human molars versus bovine incisors, did not cause any difference in the mechanical behaviors of these two types of dentin. Therefore, the anisotropic mechanical properties of coronal dentin were not affected by tooth type.

**Dependence of tensile strength of dentin on dentinal tubule orientation**

When under the same tensile loading orientation to the incremental lines (i.e., parallel or perpendicular) in human and bovine dentin, the angles between dentinal tubules and incremental lines in both types of teeth were different (Table 1). Nonetheless, tensile strengths of the parallel group (HPa and BPa) were consistently higher (p<0.05) than those of the perpendicular group (HPe and BPe). These results were obtained despite the differences in dentinal tubule orientation between human molars and bovine incisors.

Dentinal tubules and their surrounding mineralized cuffs are the most obvious structural components in dentin. However, differences in the mechanical properties of dentin are now confirmed to be related to the orientation of the collagen fibrils rather than the orientation of the tubules. In other words, results of this study contradicted the suggestion that dentinal tubules play a direct role in affecting the tensile strength of dentin.

Dentin is proved to be anisotropic. Findings of this study demonstrated that the anisotropic mechanical properties of dentin were dominated by the orientation of collagen fibrils rather than the orientation of dentinal tubules.

**Dependence of tensile strength of dentin on the orientation of incremental lines**

In a previous study, we reported that the tensile strength of dentin exhibited anisotropy according to the orientation of dentinal tubules in the tooth: tensile strength was greater when tensile loading was applied parallel to the orientation of the dentinal tubules than when applied perpendicularly. This observation agreed with the report by Bedran-de-Castro et al., but contradicted the results of other studies.⁷⁻¹⁰.

The above inconsistencies probably stemmed from differences in the banding patterns of incremental lines, because of tooth type and position within the tooth. Banding patterns of incremental lines in incisors differ from those in molars. This meant that when the direction of tensile loading was parallel to the orientation of dentinal tubules in incisors, it was nearly parallel to the incremental lines (collagen fibrils). On the other hand, when tensile loading direction was parallel to the dentinal tubules in molars, it was nearly perpendicular to the incremental lines (collagen fibrils). Therefore, the tensile strength of dentin is site-dependent, as a function of the orientation of incremental lines to tensile forces.

**CONCLUSIONS**

Within the limitations of the current study, the following conclusions were drawn:
1. Collagen fibrils, which are oriented parallel to the incremental lines, dominated the anisotropy behavior of tensile strength in coronal dentin.
2. In both human and bovine coronal dentin, tensile strength was higher when tensile loading direction was parallel to the orientation of incremental lines than in a perpendicular direction.
3. Incisor dentin is weaker along the long axis of tooth, thus rendering the anterior teeth more susceptible to subgingival fractures of the crown.
4. Molar dentin is weaker when transverse to the long axis of tooth, thus rendering posterior teeth more susceptible to cusp fractures.

Results and conclusions of this study were consistent with observations of clinical catastrophic events caused by occlusal stresses. Findings of this study suggested that the type of fractures in coronal dentin is dependent on tooth type.

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