Effect of Tubule Orientation and Dentin Location on the Microtensile Strength of Bovine Root Dentin

Jia LIU, Masayuki HATTORI, Koji HASEGAWA, Masao YOSHINARI, Eiji KAWADA and Yutaka ODA
Department of Dental Materials Science, Tokyo Dental College 1-2-2, Masago, Mihama-ku, Chiba 261-8502, Japan

Received December 7, 2001/Accepted March 26, 2002

To investigate the mechanical properties of root dentin and to further clarify the cause of vertical root fracture (VRF), this study evaluated the effect of tubule orientation (parallel, perpendicular and oblique to the cross-section of dumbbell specimens in microtensile tests) and dentin location (cervical, middle, and apical location of the root) on the microtensile strength of bovine root dentin. Each specimen was stressed in tension at a crosshead speed of 1.0 mm/min. The results of the microtensile strength measurements were statistically analyzed with one-way ANOVA and the Fisher PLSD. The oblique group (95.18±23.80 MPa) was significantly (p<0.01) higher than the parallel group (38.93±5.28 MPa) or the perpendicular group (32.64±4.69 MPa). There were no significant differences among the different dentin locations within the parallel group (p>0.05). It was clarified that the VRF occurs frequently in practical situations due to the tubule orientation of root dentin.

Key words: Vertical root fracture, Dentinal tubule, Microtensile strength

INTRODUCTION

The vertical root fracture (VRF) in endodontically treated teeth occurs frequently in clinical situations. Many clinical factors can affect its occurrence, such as traumatic occlusion, gutta-percha compression or insertion during endodontically treatment. In addition, the various structural components of root dentin can also affect the failure mode or the fracture line of VRF. However, as for the treatments of VRF, there was no reliable method for treating fractured teeth completely. Recently, advances in adhesive techniques of dentin1,2) have been successful for bonding and repairing fractured roots3). However, the complex biological structure of root dentin appears to influence the bond strength of the repaired root. Subsequently, the tensile strength of root dentin is considered important in understanding not only for the characteristics of root dentin but also the high occurrence of VRF. In addition, the more detailed information of dentin structure is essential for interpreting data from investigations on bond theory.

Dentin is a complex biological structure. The various structural components (tubule orientation or dentin location) of dentin could directly affect the mechanical properties of dentin. Tubule density, diameter and orientation vary from location to location4-8). The variation in morphology and distribution will vary considerably
over the characteristics of dentin. The specific information of tubule orientation and location is also not well established for the strength and elastic properties of dentin. Some studies examined the relation of tubule orientation with tensile strength or shear strength of crown dentin. However, studies of root dentin are few.

Currently, as a substitute for human teeth, bovine teeth are most frequently used in tests of adhesion between restorative and dental hard tissues. Some studies examined the relation of tubule orientation with tensile strength of human root dentin. However, the tubule orientation, number and size vary widely between human teeth and bovine teeth. It is, therefore, necessary to understand bovine root dentin more fully.

The methods of the microtensile test were reported to be capable of examining or testing the stress distributions more accurately than conventional methods. In addition, the small-area testing method permits multiple specimens to be prepared from one tooth. This method has been used worldwide to evaluate regional differences in the strength of dentin.

The purpose of the present study was to investigate the influence of tubule orientation (perpendicular, parallel and oblique) and dentin location (cervical, middle and apical) on the microtensile strength (MTS) of bovine root dentin.

**MATERIALS AND METHODS**

*Microtensile dumbbell sample preparation*

Eleven freshly extracted bovine incisor roots were cut longitudinally in the buccolingual direction with a low-speed diamond saw (No.11-4254, Buehler, USA) under copious flowing water to obtain distal, mesial dentin-slices and buccal dentin-slabs (thickness=1.0 mm). The natural distribution of tubule orientation at different locations of root reported that the orientation was almost oblique to the longitudinal axis and nearly parallel with each other on the center axial-interfaces. To ensure proper tubule orientation of different specimens, the tubule distributions of each specimen were observed using a stereomicroscope (×30) prior to preparation of the specimens. From the middle location of each root, the parallel, perpendicular and oblique tubule orientations on the cross-section of dumbbell specimens were randomly selected and the three groups of specimens were prepared from the mesial dentin-slices, buccal dentin-slabs and distal dentin-slices, respectively. The relation of the direction of specimens and the longitudinal axis of the root are showed in Fig. 1. As for the direction of tensile force, the tubules were at an angle of 90°, 0° and an acute angle (θ <90°) to the tensile force in the parallel, perpendicular and oblique specimens, respectively. Within the mesial dentin-slice, the cervical and apical locations were also obtained with the middle one together as the three subgroups of the parallel specimens. As in the above method, five dentin specimens were obtained from each root.

The specimens were set up with two pairs of acrylic jigs using cement materials in a special brass mold. The span between the acrylic jigs was set at 3.0 mm.
Fig. 1 Different tubule orientations and dentin locations. From the middle location of each root, the parallel (Pa), perpendicular (Pe) and oblique (O) tubules on the cross-section (⋯) of dumbbell specimens were selected, in which the tubules were at a angle of 90°, 0° and an acute angle to the direction of the tensile force (→), respectively. Within the mesial dentin-slice, the cervical and apical locations were also obtained with the middle one together as the three subgroups of the parallel specimens.

Fig. 2 Form and dimension of the dumbbell specimen. The width of the dentin slabs were inadequate for the 3.0 mm span, an acrylic plate with the same thickness (1.0 mm) as the dentin slab was used to ensure this unanimous size, which was bond to the opposite sides of the dentin slabs. Then, the microtensile dumbbell specimens with rectangular cross-sections (1.0×1.5 mm) were prepared using a fine diamond bur (Diabur, SF-12, Mani Inc., Japan) with a dental handpiece under copious air-water spraying. The sections of dumbbell portion were trimmed and shaped as shown in Fig. 2.
**Microtensile strength measurements and statistical analysis**

The microtensile dumbbell specimens were set onto a universal testing machine (RTC-1150, Tensilon, Japan) and subjected to tensile force at a crosshead speed of 1.0 mm/min. The maximum fracture force was recorded. After the tensile test, the fractured area of each specimen was more accurately measured with a digital micrometer at an accurate level of 10^-3 mm for calculating the tensile stress. One-way analysis of variance (ANOVA) was used to assess the effect of each tubule orientation and dentin location on the microtensile strength of the root dentin. Differences in dentin-location within the parallel and tubule orientations group were analyzed with the Fisher PLSD (protected least significant difference).

**SEM observations of fractured surface**

The fractured surface was cleaned by distilled water. After storing in silica gel about for 24 hrs, the fractured surfaces of different specimens were Au-Pd sputter-coated and observed using a field emission scanning electron microscope (JSM-6340F, JEOL, Japan).

**RESULTS**

**Microtensile strength measurement**

The mean microtensile strengths and standard deviations for each tubule orientation and dentin location are given in Table 1. For the tubule orientation, the oblique group, in which the tubule orientation was oblique to the cross-section of the microtensile dumbbell specimen, showed the highest strengths of the three groups (p <0.01). There was no significant difference (p>0.05) between the parallel and perpendicular groups. In the comparison of the effects of dentin location within the parallel specimens, no significant differences (p>0.05) were seen in the cervical to apical bovine root dentin.

**SEM observations of fractured surface** (Fig. 3)

The fractured lines of all of specimens were in the central part of dumbbell specimens but with different characteristics. The failure pattern showed that the fractured surface of all parallel specimens was flat and with a straight fracture-line on the side view, thus, they fractured mostly along the cross-section of the dumbbell specimens. On the flat fractured surface itself, tubules were completely fractured.

<table>
<thead>
<tr>
<th></th>
<th>Parallel</th>
<th>Perpendicular</th>
<th>Oblique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical</td>
<td>36.84±5.67</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Middle</td>
<td>38.93±5.28</td>
<td>32.64±4.69</td>
<td>95.18±23.80*</td>
</tr>
<tr>
<td>Apical</td>
<td>40.52±5.94</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

The mark (*) indicates a significant difference (p<0.01) from the others.
Fig. 3 SEM appearance of fractured surfaces (×1,000; Scale-bar. 10 µm)
Parallel tubules were completely fractured longitudinally in the parallel specimens. The round orifices of tubules appeared in the perpendicular specimens. Oblique tubules and irregular fractured dentin-steps are shown on the fractured surface of oblique specimens.

DISCUSSION

Bovine permanent incisors are commonly used in tests of adhesion between restorative materials and dental hard tissues. However, there is some concern about whether findings obtained from bovine teeth can be applied to human teeth and to the clinical situation\(^{19,20}\), because bovine incisor root dentin appears less suitable due to its significantly higher tubule density\(^4\). In addition, there may be other causes, for example differences in the relative amounts of intratubular and intertubular dentin\(^{21}\) or the nature of the intertubular matrix\(^22\). Some researchers\(^{23,24}\), concluded that bovine dentin was a satisfactory substitute for human dentin, because it was possible to use bovine teeth to better standardize the test procedure and help negate the large variations between teeth. Therefore, more detailed information of the bovine root will give a better understanding of the in vivo test substitute for human teeth.

In the conventional preparing methods of specimens, it has been difficult to establish the role of tubule orientation and morphology on the strength of dentin\(^{12}\). In 1994, Sano et al.\(^{13,17}\) developed the microtensile test method using very small surface areas, and subsequently, this method has been used in worldwide\(^{25–30}\). This test method improves stress distributions during the tensile force\(^{31}\). In addition, the microtensile test method permits multiple specimens to be prepared from one
tooth\textsuperscript{16}, and it is capable of controlling the test factors in a very small area. Therefore, different dentin locations could be obtained from the same bovine permanent incisors root specimen in the present study. A recommended surface-area of 1.6-1.8 mm\textsuperscript{2} was reported to reduce the scatter of results because this interface of small specimens also had a better stress distribution during loading\textsuperscript{32}. On the other hand, as for the cross-sectional shape of dumbbell specimens, no regulation between the cylindrical and rectangular shapes was determined\textsuperscript{33}. Therefore, in the present study, the area of 1.5 mm\textsuperscript{2} with a square cross-section of dumbbell specimens was used. In addition, the tubule orientation could be controlled with similar depths to the pulp cavity of the root from multiple dentin specimens obtained from each root.

The number and the diameter of their dentinal tubules per fractured area, in addition to the relative amount of peritubular and intertubular dentin\textsuperscript{34,35} affected the strength of the dentin. As for the variation in the number and diameter of bovine root dentin tubules, previous studies\textsuperscript{4,36} reported that there was no significant difference in the distance from the pulp surface of the root wall. Therefore, it appears that there was a homogeneous stress-distribution and deformation from inside to outside in the 1.5 mm\textsuperscript{2} cross-sections during the tensile test. In the present study, it was crucial to avoid other affecting variable for a better evaluation of the location factor (from the vertical to the apical root, but not from inside to outside in the root dentin).

As the result of the microtensile test, Table 1 shows that the oblique specimens had significantly stronger tensile strength than the parallel or perpendicular specimens. The oblique specimen findings were similar to the reported by Sano et al.\textsuperscript{13} about human crown-dentin, and the findings of Rasmussen et al.\textsuperscript{37} that the greatest resistance to fracture occurred at a small angle to the tubule direction (14°-30°). This result should contribute to the stress scattering along the dentin tubule during loading; thus, the facture stress is difficult to concentrate at a uniform mode and as a result shows a regular and flat fractured surface. In the case of root tubule orientation, this acute angle was observed previously\textsuperscript{18}, where tubules were arranged approximately radioactively around the pulp and angled at a mean of 12.5° ± 4.8° to the root face in the tooth's transverse axial plane. According the method of specimen preparation method in the present study, the stress scattering had a practical role in the oblique specimens, in which tubules ran at an acute angle to the applied tensile force. As a consequence, a very rough fractured surface was shown in the failure mode of the oblique group. In contrast, there was no stress scattering in the case of parallel and perpendicular specimens because the direction of the tubules was at an angle of 90° or aligned with the tensile force (0°). Consequently, the tensile force could be concentrated more easily at the center of the interface of dumbbell specimens in the parallel and perpendicular specimens. Due to this action of stress concentration or scattering, flat fractured surfaces were exhibited in all parallel specimens and most perpendicular specimens.

The microtensile strength of the parallel specimens was greater than that of the perpendicular specimens, even with no significant difference in this study. This is
consistent with previous findings that dentin is easiest to fracture across dentinal tubules. A highly mineralized cuff of peritubular dentin surrounds the tubule lumen, and the tubules are separated by intertubular dentin composed of a matrix of type-1 collagen reinforced by apatite. The peritubular dentin may be more properly termed intratubular dentin and contain mostly apatite crystals with little organic matrix. The result comparing the parallel and perpendicular groups in this study may have contributed to the different mechanical properties of the peritubular dentin and intratubular dentin. It also may be explain by the orientation relationship of collagen (and apatite crystal) and tubules. Further studies on the role of peritubular dentin and intratubular dentin while under a tensile force are required to clarify this matter.

The highest incidence of about 10% or 30% of VRF occurs in teeth after endodontically treatment. The causes were reported as dehydration of the hard structure and dentinal tubules with a moisture loss of 9% or an extensive enlargement of the root canal and condensation stress or posts and crowns prosthetics after endodontic therapy, and so on. An important intrinsic factor of tubules is that they are arranged approximately radioactively around the root pulp in the tooth’s transverse plane, and run nearly parallel with each other in the central axial interfaces. The highest incidence of VRF may be associated with the original tubule orientation of root dentin. According to the results of this study, it was shown that the fracture resistance of parallel and perpendicular tubules to tensile stress was significantly weaker than that of oblique tubules. A fractured line usually progresses into the weaker fracture resistance area. Therefore, this study can help further clarify the high incidence of VRF, where the crack will easily progress into the interface of the parallel or perpendicular tubules and then form vertical but not horizontal fracture line in clinical situations.

In addition, no significant influence of root dentin location on the tensile strength of the parallel specimens was found, even with the increasing trend from the cervical to the apical root dentin. The no significant may have contributed to the diameter of the tubules and can attributed to the bovine root dentin which showed no significant difference from the vertical to apical root. This result suggested that the method of specimen preparing in this study permits multiple specimens (about 8-10 specimens) to be prepared from one root with nearly similar original tensile strength. It is very useful for consistency of material properties and standardizing the original conditions of specimens more easily. It is difficult to improve knowledge about the complex biological structure of bovine or human teeth, in concert with the original situation from the individual intrinsic factors such as the age, position of teeth in oral cavity, calcium concentration, caries free or affected, and so on. However, based on the findings of the present study about the characteristics of dentin, one useful method was indicated where multiple specimens could be obtained from the cervical to apical position of one root, which showed no significant difference in resistance to tensile force, and that parallel tubules were showed on the fractured surfaces as similar as the situation of VRF. For future adhesive studies of bonded
VRF, it is possible to avoid heterogeneity and to standardize the intrinsic factors of root dentin to compare the various adhesive conditions using multiple specimens from one root.

A tapered tip tool\cite{46,47} is typically used to half the root embedded in acrylic resin for simulating and investigating a VRF or a bond-repaired VRF in conventional methods. With this method, it is difficult to calculate the fractured area. However, the micro-dumbbell specimen method in the present study allowed correct controlling and measurement of the area (for the flat fractured surface) and the tubules orientation to the applied force at different locations. It is better suitable for imitating local situations of repaired VRF. This method also allows the possibility of obtaining percentage data to compare the fracture resistance of intact root dentin and the bond strength of VRF by using the same specimen after one facture load.

CONCLUSIONS

According to the investigation of the effect of tubule orientation and dentin location on the microtensile strength of bovine root dentin, it was revealed that there was a significant influence by the factor of tubule orientation, but not in the case of dentin location. In the tensile stress examination, root dentin was easier to fracture in a parallel or perpendicular orientation to the tubules than that in an oblique orientation. Therefore, it appears that the VRF occurs frequently in practical situations due to the tubule orientation of the root dentin.

Using the specimen preparing method in this study, it is possible to obtain multiple specimens from one root to avoid heterogeneity and to standardize the intrinsic factors of root dentin for simulating and investigating a VRF or a bonded VRF. This method can obtain percentage data to compare the fracture resistance of intact root dentin and the bond strength of an adhesively repaired VRF using the same specimen after one facture load.

ACKNOWLEDGEMENTS

This investigation was supported in part by a Grant-in-Aid from Japan-China Medical Association and Futaba Corporation. We thank Sun Medical Ltd. for providing materials.

REFERENCES


