Comparison of nanohardness between coronal and radicular intertubular dentin

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This study investigated the hardness and Young's modulus of coronal and radicular intertubular dentin. Ten bovine teeth were each divided into coronal and radicular groups, and the flat surfaces of the coronal and radicular dentin were subsequently processed along the tooth axis. The hardness and Young's modulus of the coronal and radicular intertubular dentin were evaluated using nanoindentation tests, at two locations per tooth. Mean hardness and Young's modulus values were statistically compared by one-way ANOVA and Fisher's PLSD test. The hardness of coronal intertubular dentin was 0.81 ± 0.05 GPa and that of radicular dentin was 0.55 ± 0.02 GPa. Additionally, the Young's modulus of coronal intertubular dentin was 26.60 ± 2.19 GPa and that of radicular dentin was 20.89 ± 1.10 GPa. Findings of this study revealed that the hardness and Young's modulus of coronal intertubular dentin were greater than those of radicular intertubular dentin.

Keywords: Dentin, Hardness, Intertubular dentin

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INTRODUCTION

The understanding of the mechanical properties of dentin is very important for clinical diagnosis and in the evaluation of dental materials when considering the design or lifetime duration. Dentin is covered by enamel tissues, and coronal dentin is the interface between enamel and radicular dentin. Radicular dentin, in turn, is the interface between the bone and coronal dentin¹⁾. Thus, dentin is constituted of coronal dentin and radicular dentin.

It has been reported that fracture behaviors between coronal and radicular dentin are different as indicated by strain concentration tests and impression-induced damage tests²⁾. In clinical dentistry, microtensile bond strengths are different between coronal and radicular dentin^{3,4)}. Moreover, the ultimate tensile strength of coronal dentin differs from that of radicular dentin along the tooth axis. Tensile strength of radicular dentin is greater than that of coronal dentin⁵⁻⁷⁾. Tensile tests using dumbbell-shaped specimens are reportedly useful for identifying defects easily^{5,8-12)}. The fracture surfaces of coronal dentin are different from those of radicular dentin as observed by scanning microscopy after tensile tests. In particular, the fracture surfaces of coronal intertubular dentin are smooth whereas those of radicular intertubular dentin are rough⁵⁾.

However, a large sample size is needed when performing tensile tests, and dentinal tubules are

known to vary in length between specimens¹³⁾. Additionally, dentinal tubules are composed of peritubular and intertubular dentin. It has been reported that the mechanical properties of dentin are dominated by those of intertubular dentin¹⁴⁾. Therefore, it is important to consider the mechanical properties of intertubular dentin.

Reports are scarce when it comes to comparing the mechanical properties of coronal and radicular intertubular dentin. Hence, the aim of this study was to determine and compare the nanohardness and Young's modulus of coronal and radicular intertubular dentin. Indentation is widely used to evaluate hardness and Young's modulus for dentin^{1,15-23)}. This is because nanoindentation results in very small indented regions, making it possible to clarify the intertubular dentin properties irrespective of peritubular dentin or the dentinal tubules.

MATERIALS AND METHODS

Materials

Bovine lower central incisors were extracted immediately after sacrifice from the animals assumed to be 2 to 2.5 years old according to their dental age. Fractured or poorly mineralized teeth were not included. Teeth were frozen within 3 hours of extraction and stored at -12°C in a freezer until use. Before preparation, the teeth were defrosted by immersion in Hank's balanced salt solution at room

Fig. 1 (A) Schematic representation of the location of dentin slab in bovine tooth.
(B) The positions of coronal and radicular dentin for hardness and Young's modulus measurements.

temperature for 30 minutes.

Preparation of specimens

Ten bovine teeth were used in this study. A 3-mm-thick dentin slab was harvested from each tooth using a low-speed cutting machine (Isomet, Buehler, Lake Bluff, IL, USA) under copious water cooling, whereby each slab was cut along the plane containing the incisal edge and the apex of the root (Fig. 1A). Next, the dentin slabs were sectioned into coronal and radicular portions using a diamond cutting disk at the cemento-enamel junction. The coronal and radicular dentin of each tooth were embedded in acrylic with an epoxy resin (Palapress vario, Heraeus Kulzer GmbH, Hanau, Germany). The labial sides of the coronal and radicular dentin were prepared for indentation.

After sectioning, the specimens were sequentially polished with 600-, 800-, 1000-, 1200-, 1500-, and 2000-grit silicone carbide (SiC) papers (Maruto Instrument Co. Ltd., Tokyo, Japan), each for about 5 minutes under wet conditions. Final polishing was performed on each specimen with 15-, 9-, 6-, 3-, 1-, and 0.1- μ m diamond lapping films sequentially, each for about 20 minutes under wet conditions (Buehler, Lake Bluff, IL, USA). After polishing each segment, the smear layer and debris were removed by ultrasonic cleaning for 5 minutes. The polished specimens were stored in Hank's balanced salt solution at 4°C prior to use.

Hardness and Young's modulus measurements

The positions for hardness and Young's modulus

Fig. 2 Schematic representation of the load *versus* indenter displacement curve for an indentation experiment.

measurements are indicated in Fig. 1B. There were two focus areas, namely coronal dentin and radicular dentin. The indentation positions were as follows: the coronal area was 1.5 mm midway from the DEJ along the line including the edge of the coronal dentin and the center of the pulp chamber wall; the radicular area was 1.5 mm midway from the outer surface and 4 mm below the CEJ. A total of five triangular indentations were determined and recorded at different segments on the intertubular dentin of each tooth. The average data for each tooth was then taken from the five indentations, which were done at a distance of 10 μ m from each other. The hardness and Young's modulus results of the specimens were statistically analyzed by one-way ANOVA and Fisher's PLSD test (significance was accepted at $p < 0.05$).

To measure hardness and Young's modulus, a dynamic hardness tester (DUH-W201, Shimadzu Corp., Kyoto, Japan) attached to a Berkovich indenter, a three-sided pyramid diamond probe, was used. Room temperature was maintained at $26 \pm 0.5^\circ\text{C}$. Each specimen was placed on the stage of the tester with the specimen surface orientated parallel to the stage. The tip then approached the specimen at a velocity of 1 mN/s. Hardness was measured using a force of up to 5 mN to create a superficial indent on the specimen surface. After contact had been established, pressure was applied for 5 seconds.

During indentation, a curve describing the relationship between load P and displacement h was continuously monitored and recorded (Fig. 2). The

mechanical properties of the material could be derived by analyzing the load-displacement data during the loading-unloading indentation cycle. Hardness, H , is given by Eq. (1) as follows:

$$H = 37.8 \times P_{\max} / h_{\max}^2 \quad (1)$$

where $P_{\max}$ is the maximum applied load and $h_{\max}$ is the indenter displacement at maximum load.

To determine Young's modulus from the force displacement data, the procedure outlined by Oliver and Pharr²⁴⁾ was followed. Young's modulus, E , is given by Eq. (2) as follows:

$$E = (dP / dh) (\sqrt{\pi} / 2\sqrt{A}) \quad (2)$$

where dP / dh is the slope of the unloading curve at maximum force and A is the contact area created by the indentation.

Scanning electron microscope (SEM) observations and elemental analysis of coronal and radicular dentin

The specimens were coated to a thickness of 200 Å in a carbon coater (VC-100, Vacuum Device Inc., Ibaraki, Japan) after the indentation test. The indentations of each specimen were then observed using a scanning electron microscope (S-4500, Hitachi Ltd., Tokyo, Japan). A backscattered electron image was filmed using yttrium-aluminium-garnet (YAG) backscattered electron imaging and at $\times 2000$ magnification.

Additionally, an energy dispersive X-ray spectroscope (EMAX-7000, Horiba Ltd., Tokyo, Japan) was used to determine the elemental composition of each area. Average data of each tooth was obtained from the five sets of data near each indentation. In this manner, the Ca, P, and Mg concentrations as well as the Ca/P ratio in coronal and radicular dentin were determined. The measurement area for these concentrations was $2 \mu\text{m} \times 2 \mu\text{m}$, which was approximately the same as the hardness measurement area.

RESULTS

Hardness and Young's modulus measurements of coronal and radicular dentin

Figures 3A and 3B show the hardness and Young's modulus results of coronal and radicular intertubular dentin, which were obtained at two locations of each tooth using nanoindentation tests. The mean hardness and Young's modulus values were compared statistically by one-way ANOVA and Fisher's PLSD test (significance was accepted at $p < 0.05$).

Hardness of coronal intertubular dentin was 0.81 ± 0.05 GPa, while that of radicular dentin was 0.55 ± 0.02 GPa. The Young's modulus of coronal

Fig. 3 (A) Hardness values of coronal and radicular dentin ($n=10$). (B) Young's moduli of coronal and radicular dentin ($n=10$). (C, D) Typical indentation images after the indentation test.

Fig. 4 Elemental compositions of coronal and radicular dentin. (A) Ca in wt%; (B) P in wt%; (C) Mg in wt%; (D) Ca/P ratio.

Vertical lines represent SD. The horizontal lines above the bars indicate that groups differ statistically from each other ($p < 0.05$).

intertubular dentin was 26.60 ± 2.19 GPa, while that of radicular dentin was 20.89 ± 1.10 GPa. Results showed that the hardness and Young's modulus of coronal intertubular dentin were greater than those of radicular intertubular dentin.

SEM observations and elemental analysis of coronal and radicular dentin

The indentations on the specimens following the nanoindentation tests were confirmed using a microscope with a CCD camera under $\times 700$ magnification, as indicated in Fig. 3C. Arrows showed the indents in the intertubular dentin. Typical indentation cracks both of the coronal and radicular dentin were not seen in the scanning electron microscope images (Fig. 3D).

On elemental analysis, the obtained mineral contents of each group are indicated in Figs. 4A–D. The amount of Ca in coronal dentin was 41.22 ± 0.88 wt%, while that in radicular dentin was 38.18 ± 0.61 wt%. The P content in coronal dentin was 22.52 ± 0.24 wt%, while that in radicular dentin was 22.01 ± 0.31 wt%. The amount of Mg in coronal dentin was 0.67 ± 0.09 wt%, while that in radicular dentin was 1.37 ± 0.11 wt%. The Ca/P ratio (MR) of coronal dentin was 1.43 ± 0.02 , while that of radicular dentin was 1.34 ± 0.02 . Results showed that the Ca content and Ca/P molar ratio of coronal dentin were significantly higher than those of radicular dentin. In terms of P content, there were no significant differences between each dentin region. In terms of Mg content, that in coronal dentin was significantly lower than that in radicular dentin.

DISCUSSION

There have been many reports on dentin hardness over the past 70 years^{25–32}. It is well known that the hardness of dentin varies depending on the area^{25–28}. Previous studies have used indentations to measure

hardness. However, owing to the largeness of the indentation areas, measurements included not only intertubular dentin but also dentinal tubules and peritubular dentin. Consequently, the hardness values were merely a composite average.

The difference in hardness between coronal and radicular dentin stems from their differences in structure, i.e., tubular density, whereby it has been reported that tubular density differs between coronal and radicular dentin³³. As tubular density is closely correlated with the position between coronal and radicular dentin, it is not possible to determine from microindentation measurement methods whether decreased hardness is due to dentin morphology (increased tubular density) or due to differences in the material properties of the constituents of dentin. Incidentally, dentin consists of peritubular dentin, intertubular dentin, and dentin lumens.

Kinny *et al.* investigated the hardness of intertubular and peritubular dentin at two locations, near the dentino-enamel junction and near the pulp in coronal dentin¹³. They confirmed that the hardness of intertubular dentin near the dentino-enamel junction was greater than that of intertubular dentin near the pulp. The hardness of intertubular dentin, but not that of peritubular dentin, differs depending on the site. Previous study has already reported that the Knoop hardness of radicular dentin was significantly lower than that of coronal dentin in bovine incisors². Similarly in the present study, results showed that the hardness and Young's modulus of coronal intertubular dentin were greater than those of radicular intertubular dentin. In other words, our results were in agreement with those of previous study². We also confirmed that intertubular dentin exhibited statistically significant differences in hardness and Young's modulus according to the site under investigation. Table 1 shows the reported values of hardness and Young's modulus of dentin, where the hardness of intertubular dentin ranged

Table 1 Reported values of hardness and Young's modulus of dentin

Type of teeth	Site	Hardness (GPa)	Young's modulus (GPa)	Authors and dates
Third molar	Coronal intertubular		20	Kinny JH <i>et al.</i> (1999) ¹⁴
Premolar and canine	Coronal intertubular	0.4–0.7	18–27	Hosoya Y <i>et al.</i> (2005) ¹⁵
Premolar	Coronal intertubular	0.7	21	Chung HK <i>et al.</i> (2005) ¹⁸
Third molar	1mm superior to the cervical margin	0.8	20–24	Kinny JK <i>et al.</i> (2003) ¹⁹
Premolar	Coronal intertubular	0.9	15	Poolthong S <i>et al.</i> (2001) ²⁰
Third molar	Coronal intertubular	0.8–1.0	18–21	Marshall GW <i>et al.</i> (2001) ²¹
Third molar	Coronal intertubular	0.8	20	Marshall GW <i>et al.</i> (2001) ²²
Root	Radicular intertubular	0.5–0.8	24–25	Balooch M <i>et al.</i> (2001) ²³

from 0.4 to 1.0 GPa and that the Young's modulus ranged from 18 to 27 GPa. In the present study, our data were also within the range of these reported values.

Hardness tests measure the resistance of dentin to deformation caused by the penetration of an indenting stylus. This technique measures the indenter load as a function of depth of penetration from which the contact stiffness is obtained from the derivative of the unloading curve evaluated at the peak force. The modern theory of indentation, where force and stylus displacement are analyzed to measure Young's modulus, has been applied to mineralized tissues only in the last decade. The elastic properties of dentin are important for understanding the mechanical properties of calcified tissue. For this reason, nanoindentation has recently become a common technique for the determination of local mechanical properties of structural features in biological hard tissues.

To compare the fracture surfaces of coronal and radicular dentin, the tensile test has been used. It was previously shown that the collagen fibers in dentin contributed to the tensile strength of dentin^{6,10}. The amount of collagen fibers in radicular dentin is greater than that in coronal dentin³⁴. Therefore, the tensile strength of coronal dentin is lower than that of radicular dentin. As for dentin hardness, it is related to the mineral content¹⁴. To date, there are no reports comparing the degree of mineralization of intertubular dentin between coronal and radicular dentin¹⁴. Against this backdrop of information scarcity, we compared the mineral components of coronal and radicular dentin and showed that the Ca/P ratio of coronal intertubular dentin was higher than that of radicular intertubular dentin. The Ca/P ratio is closely correlated with the hardness value^{35,36}. On this premise, we also confirmed that the mineral content contributed to the hardness of dentin.

The hardness and Young's modulus of dentin might be affected not only by region/location, but also by a multiplicity of factors such as animal species (e.g., human or bovine), type of tooth (e.g., molar or incisor), and human characteristics (e.g., age, sex, race, or environment). These variations need to be taken into account and studied in depth to the end of obtaining useful insight for clinical science.

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