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Micro-tensile strength of sound primary second molar dentin

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Although biomechanical properties of dentin are important factors to dentin bonding, as well as for understanding caries, cervical erosion/abfraction, and tooth fracture, limited information for primary teeth has been reported. This study evaluated the micro-tensile strength (MTS) of sound primary second molar dentin with an originally designed system that we have developed. Twenty-seven dumbbell-shaped specimens were prepared from eight teeth. The MTS of the dentin beneath the occlusal surface was measured and fractured dentin surfaces were observed using SEM. Data was analyzed using ANOVA subsequent to Fisher's PLSD at $p < 0.05$. The novel jig system used in this study allowed symmetric dumbbell-shaped and uniformly sized specimens. The mean (standard deviation) MTS of all the specimens was 38.2 (15.9) MPa. The mean MTS of the specimens sectioned from the central area (46.5 MPa) was significantly higher than those of the specimens that were sectioned from the most mesial (31.1 MPa) and distal (27.8 MPa) sides of the teeth. Sound primary second molar occlusal dentin showed regional variations in tensile strength. This might influence the prognosis of dental restorations.

1. Introduction

Dentin is a structurally anisotropic biological composite, and exhibits regional differences in mineral concentration, tubule density and diameter, and collagen orientation [1, 2]. Permeability increases, and bond strength of dental adhesive materials decreases in deeper dentin [3, 4]. Regional differences in shear strength [5, 6] and tensile strength [7, 8] have been shown for permanent coronal dentin. Hardness of permanent dentin decreases with depth [8, 9] and was inversely correlated with dentin tubule density [9], although another study [10] showed that part of the decrease may be due to changes in the intertubular dentin with depth. The influence of these regional variations on biomechanical function are important factors for dentin bonding, as well as for understanding dental caries, cervical erosion/abfraction, and tooth fracture.

Biomechanical properties and regional variations in primary (deciduous) dentin have received limited attention although substantial differences occur in its structure [11]. Prior studies of mechanical properties have focused mainly on Knoop hardness [12, 13]. Recently nano-hardness and elastic modulus have been reported [14, 15]. Matching the properties of restorative materials to the properties of teeth may be important to enhance the longevity of the dental restorations. As a consequence, baseline mechanical property data of teeth are required.

The recently developed micro-tensile test presents several advantages over conventional testing methods [16, 17]. This method can test very small surface areas,

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provides multiple specimens from one tooth and allows study of regional differences. However, it is difficult to make symmetric and uniformly sized dumbbell-shaped or hour-glass-shaped specimens for the micro-tensile strength (MTS) testing and an improved approach for sample fabrication is desirable. Although there have been many reports on MTS of permanent dentin, the MTS of primary dentin has not been reported to our knowledge.

The purpose of this study was to evaluate the MTS of sound primary second molar dentin using a recently developed method [18]. The hypothesis of this study was that there are regional differences of the MTS for primary dentin.

2. Materials and methods

2.1. Sample teeth

Eight sound primary second molars (6 maxillary, 2 mandibular) that were obtained from Japanese children were used. The primary teeth were extracted by eruption of the succedaneous permanent tooth or orthodontic treatment. The teeth were frozen immediately after extraction or exfoliation in physiologic saline containing 0.05% sodium azide until prepared. Informed consent was obtained from parents and patients for collecting teeth.

2.2. Micro-tensile test

Thirty-two specimens were sectioned using a low-speed circular diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under copious water-cooling. Sections were 1.0 mm thick and cut parallel to the long axis of the tooth bucco-lingually. Three to five specimens came from each tooth. The region of the specimens was classified into the most mesial or distal sides, while all other specimens were classified as central.

A new system was used for preparing dumbbell-shaped specimens and measuring MTS. It consisted of the TDC (Tokyo Dental College type) jig, adjuster, bur attachment and guide plate (Fig. 1A–D). The TDC jig has 4 acrylic plates. Each specimen was set on the jig so the direction of dentinal tubules would be almost perpendicular to the tensile stress. Then the specimen was sandwiched between two plates of the jig and bonded by etching only the enamel for 10 sec using 10-3 etchant and Superbond adhesive resin (Sun Medical Co., Moriyama, Japan). Specimens were kept in a 37°C dry thermostat for 15 min and then immersed in 37°C physiologic saline solution for 24 hr. The distance between the upper and lower jig plates was standardized at 3 mm using the TDC adjuster (Fig. 1A). The specimen between the plates was then placed in the specially designed TDC guide plate (Fig. 1B). The length of the fine

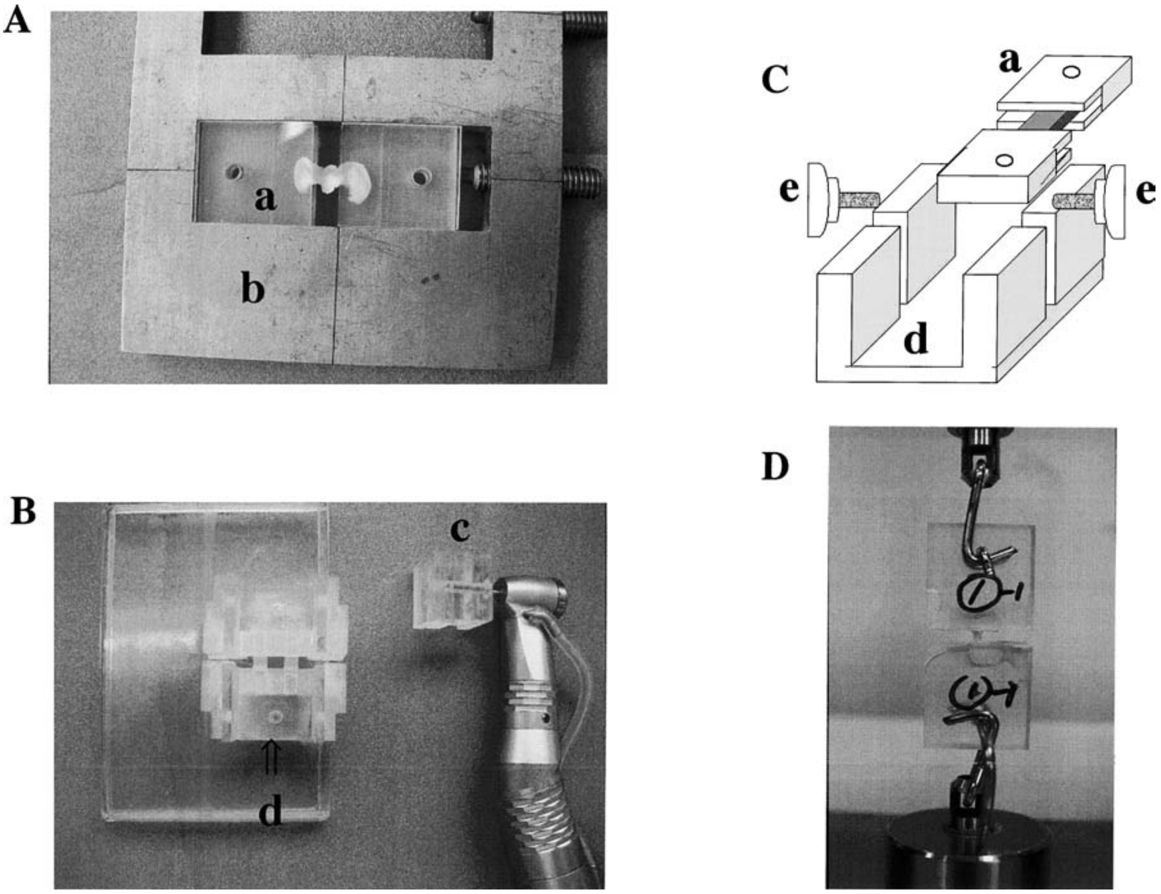

Figure 1 Novel jig system: (A) The TDC (Tokyo Dental College type) jig (a) and adjuster (b). The TDC jig has 4 acrylic plates. Specimens were placed on the jig so the direction of dentinal tubules would be perpendicular to the tensile stress. Then the specimen was sandwiched between two plates of the jig and bonded. The distance between the upper and lower plates of the jigs was standardized at 3 mm using the adjuster. (B) The TDC bur attachment (c) and guide plate (d). The specimen that was set between the jig plates was placed in the specially designed guide plate. The length of the fine diamond bur was standardized using the bur attachment. (C) Dumbbell-shaped specimen was prepared using the diamond bur (e), with shape determined by the guide plate (d). (a): TDC jig. (D) The MTS of the specimen prepared with the novel jig system was measured by a universal testing machine.

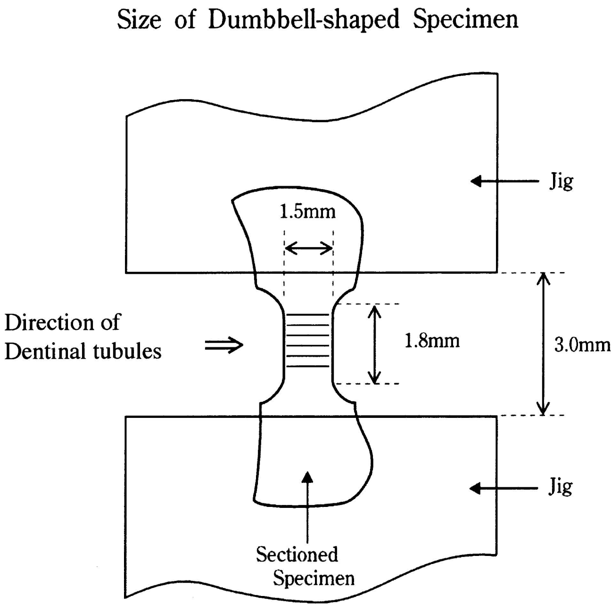

Figure 2 Size of dumbbell-shaped specimen. The bucco-lingually sectioned tooth-crown specimen was set on the TDC jig. The size of the dumbbell-shaped specimen with 1 mm (thickness) × 1.5 mm (width) × 1.8 mm (length) narrow portion.

diamond bur (SF-12, Mani Inc., Tochigi, Japan) was standardized using the TDC bur attachment (Fig. 1B). Dumbbell-shaped specimens with 1 mm (thickness) × 1.5 mm (width) × 1.8 mm (length) narrow portion were prepared from each section as shown in Fig. 2 using the diamond bur with a dental handpiece (ME16-CA13HP, Osada Co., Tokyo, Japan) under copious air-water cooling. A standard specimen shape was produced by use of the guide plate (Fig. 1C). The load to fracture of the dentin beneath the occlusal surface was measured by a universal testing machine (Tensilon RTC-1150-TSD, Onentec Co., Tokyo, Japan) at a crosshead speed of 1 mm/min (Fig. 1D). The fractured area of each specimen was measured with a profile projector (RJ-300, Mitutoyo Co., Tokyo, Japan) to calculate the MTS.

2.3. Microscopic and SEM observation

The direction of fracture was observed using a microscope (SZH, Olympus Co., Tokyo, Japan) and classified as either perpendicular or oblique to the tensile stress (Fig. 3). Then the fractured surfaces were gold sputter coated and observed by a scanning electron microscope (SEM; S-3500, Hitachi Ltd., Tokyo, Japan). The orientation of dentinal tubules on the fractured surfaces was classified as parallel, perpendicular or oblique to the direction of dentinal tubules [18]. The areas of each orientation were calculated from the SEM image of each specimen.

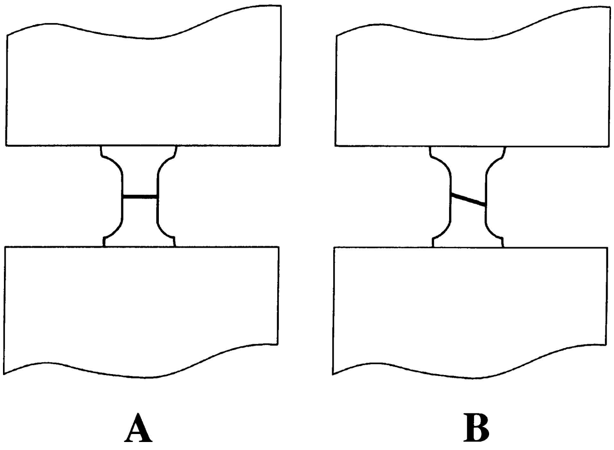

Figure 3 Direction of fracture. The direction of fracture was classified as either perpendicular to the tensile stress (A) or oblique to the tensile stress (B).

2.4. Data analysis

MTS were compared for maxillary and mandibular, different sectioned regions, and direction of fracture relative to the tensile stress and tubule orientation using ANOVA and subsequent Fisher's PLSD at $p < 0.05$. The correlation between the MTS and the orientation of dentinal tubules on the fractured surfaces was analyzed using Spearman's rank correlation coefficient at $p < 0.05$.

3. Results

Five out of 32 specimens were excluded from the data because fracture did not occur at the center of the dumbbell region.

Table I shows the MTS means and standard deviations classified by sectioned region. The MTS mean and standard deviation of all the specimens was 38.2 ± 15.9 MPa with no significant differences between maxillary (36.3 ± 12.1 MPa) and mandibular (45.0 ± 25.6 MPa) primary second molars. For all teeth, the mean MTS of the specimens from the central area (46.5 ± 15.7 MPa) was significantly higher than those of the specimens from the most mesial (31.1 ±

TABLE I Means (standard deviations) of MTS of sound primary second molars compared with sectioned regions (Unit: MPa)

	Maxillary		Mandibular		Total	
Sectioned region	Mean (S.D.)	Number of specimens	Mean (S.D.)	Number of specimens	Mean (S.D.)	Number of specimens
Central	42.4 (11.2)	10	56.7 (22.3)*	4	46.5 (15.7)	14
Mesial	31.4 (14.6)	5	29.5	1	31.1 (13.1)	6
Distal	30.1 (6.6)	6	13.6	1	27.8 (8.7)	7
Total	36.3 (12.1)	21	45.0 (25.6)	6	38.2 (15.9)	27

Vertical lines show no significant difference at $p < 0.05$.

*Since only a small number of the specimens were available, statistical difference among the sectioned regions could not be obtained.

TABLE II Means (standard deviations) of MTS of sound primary second molars compared with direction of fracture to tensile stress (Unit: MPa)

Direction of fracture	Mean (S.D.)	Number of specimens
Perpendicular	36.3 (17.3)	19
Oblique	42.7 (11.5)	8
Total	38.2 (15.9)	27

Vertical line shows no significant difference at $p < 0.05$.

13.1 MPa) or distal side (27.8 ± 8.7 MPa) of the teeth. No significant difference was found in comparisons between the most mesial and distal sides.

Table II shows the MTS means and standard deviations of the specimens compared by direction of fracture (Fig. 3). Nineteen specimens had perpendicular fracture and 8 had oblique fracture. The mean MTS of the specimens with oblique dentin fracture (42.7 ± 11.5 MPa) was higher than that of the specimens with perpendicular dentin fracture (36.3 ± 17.3 MPa) but there was no significant difference between them.

Fig. 4 shows a fractured surface of a specimen sectioned from the central region of a mandibular primary second molar. The MTS was 80.9 MPa and almost all dentinal tubules on the surface fractured parallel to the tubule direction. The number of the specimens classified with the orientation of dentinal tubules on the fractured surfaces from SEM was 2 for parallel and 25 for mainly parallel and partially oblique and/or perpendicular to dentinal tubules. Parallel fracture was the main type, and was observed on all of the specimens. The mean area percentage of parallel fracture were 85.8 ± 12.2% for maxillary, 82.5 ± 18.9% for mandibular and 85.1 ± 13.6% overall. There was no significant differences among the percentages of the parallel fracture for the central, mesial and distal areas for maxillary teeth or overall. For mandibular teeth, since only a small number of the specimens were available, statistical differences among the sectioned regions could not be obtained. No significant correlation was observed between the MTS and percentage of the fracture parallel to dentinal tubules.

4. Discussion

Tensile testing of oriented human dentin samples is technically difficult. Specimen size especially for primary teeth is limited by the thickness of dentin (maximum, about 2.5 mm), direction of dentinal tubules differs in the same tooth, and a graduation exists in both morphology and mechanical properties from the pulpal surface to outer layers of dentin [5–7, 10, 12–15, 19, 20]. Attempts to minimize specimen size have included bonding of dentin samples to the test apparatus with cyanoacrylate [16] or adhesive resin (this study) at the ends of the samples. If the length of the specimen is short, bonding material might penetrate into the dentin close to the measuring area. In this study, etching and bonding was done only on the enamel of the specimen (Fig. 1A). Since the occlusal dentin thickness of the specimens was thin, variation of MTS with depth was not measured in this study.

For tensile testing, stick-shaped, hour-glass-shaped or dumbbell-shaped specimens have been used. Hour-glass-shaped or dumbbell-shaped specimens offer the advantage of yielding fractures in the uniform reduced section at a predictable location away from the ends of the specimens. Generally dumbbell shaping has been done free-hand using a bur, and it is difficult to make symmetric-shaped and standardized specimens of equal size. The system used in this study allowed symmetric dumbbell-shaped and uniformly sized specimens. Compared to the general methods, this system offered better alignment of test direction relative to the direction of dentinal tubules. However, since the direction of dentinal tubules is various and dentinal tubules do not run straight, it was difficult to set the dentinal tubules in dumbbell portion to be perfectly perpendicular to the tensile stress.

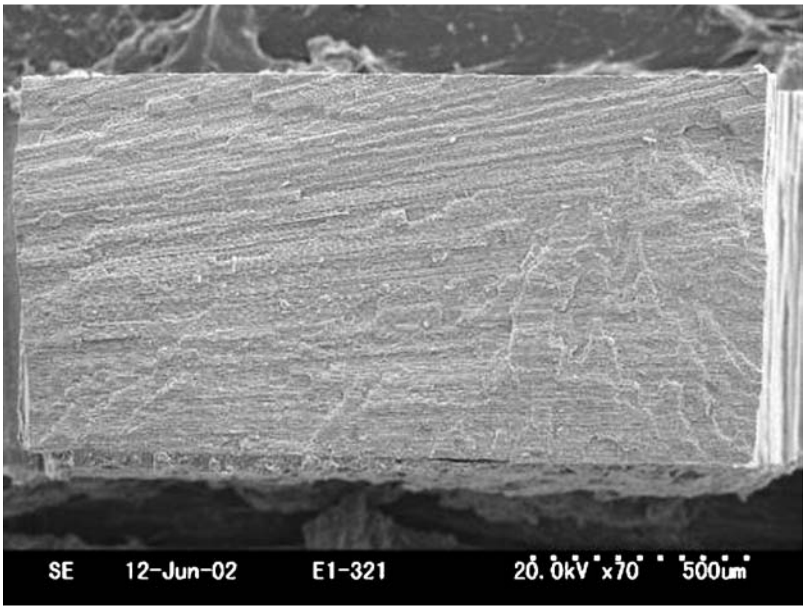

Figure 4 Fractured surface after the MTS testing. This SEM view shows a fractured surface of a specimen sectioned from the central region of a mandibular primary second molar. The MTS was 80.9 MPa and almost all dentinal tubules on the surface fractured parallel to the tubule direction.

Previously reported MTS of human third molar coronal dentin was 104 MPa [16]; while another study [7] reported inner dentin gave 44.4 MPa near the pulp, which was significantly lower than 97.8 MPa near the DEJ. When loaded perpendicular to tubule orientation, significantly higher (80 MPa) values were found than for specimens loaded parallel to tubules (58 MPa) [20]. MTS of human maxillary anterior cervical dentin were 60.3 MPa when tested perpendicular and 36.7 MPa when tested parallel to the tubules [21]. The wide variation of the data might be caused by the differences of the test method and the known effect that smaller samples give higher strength values [22].

In this study, the MTS was obtained from the dentin beneath the occlusal surface of primary molar dentin and the tensile stress (load) was applied perpendicular to tubule orientation. The MTS of this study (Table I) ranged from 27.8 (8.7) MPa in the most distal side to 46.5 (15.7) MPa in the central area. These values were lower than many of the previously reported MTS of permanent dentin, but similar to the MTS of inner permanent dentin [7] and cervical dentin when loaded parallel to tubule orientation [21]. The lower mineral content of primary dentin [23] could also result in lower hardness, modulus and result in the lower MTS, as compared with permanent dentin. However, sample area could also play a role. In this study, the size of the dumbbell narrowest cross sectional area of the specimen was about 1.5 mm^2, slightly larger than that for the previous MTS studies in which the size was 1.13 mm^2 [21], 0.5 mm^2 [20] and 0.25 mm^2 [16]. Thus the lower MTS in this study might be a result of the larger size of the narrowest cross sectional area.

Lower MTS in the inner dentin might be mainly caused by the lower hardness and modulus of inner dentin [9, 10], and the possibilities that the fracture toughness is lower near the pulp or that the flaw distribution is altered from that in outer dentin [7]. In this study, MTS was measured midway between DEJ and pulp chamber wall. However, because of the small thickness of primary dentin, the dumbbell narrowest portion might partially include the dentin near the DEJ in some specimens but include the dentin near pulp in other specimens. This could lead to the high standard deviations seen in this work. Tooth to tooth difference of the MTS also could contribute to the high standard deviations.

The mean MTS of specimens from the central area was significantly higher than those from the other regions (Table I). Previous work [18] using bovine root dentin reported that orientation of dentinal tubules on the fractured surface influenced the MTS in the oblique fracture group, in which the stress scattering had a practical role and showed a very rough fractured surface. Oblique fractures were significantly higher than either the parallel or perpendicular fracture group. In this study, most specimens showed mainly parallel tubule fracture (Fig. 4) and there was no significant difference of the tubule orientation pattern among the sectioned regions. In this study, eight (5 specimens from center region, 2 specimens from most mesial region and 1 specimen from most distal region) out of 27 specimens had oblique fracture to the tensile stress (Fig. 3). However, the tubule orientation pattern on all the fractured surfaces of these specimens was mainly parallel to the dentinal tubules and there was no significant difference of the MTS between the perpendicular and oblique fractured specimens (Table II). For these specimens, direction of dentinal tubules in the narrow dumbbell portion might not be perpendicular but slightly oblique to tensile stress but fracture occurred parallel to dentinal tubules and caused oblique fractures as shown in Fig. 3. In the most mesial and distal sides, the direction of the dentinal tubules were not prepared perfectly parallel to the long axis of tooth and they differed from the central area where the dentinal tubules were relatively parallel to the long axis of tooth. This factor might influence the MTS.

Although no significant difference could be detected, probably due to the limited numbers of specimens and the large standard deviations, the specimens with oblique dentin fractures (Fig. 3) had higher mean values than the samples that fractured perpendicular to the stress (Table II). A significant effect of tubule orientation on tensile strength has been reported [20, 21, 24]. It is suggested that this result might be explained in relation to collagen (and apatite crystal) orientation [21], or because the tubules act as voids [24].

We concluded that the results of this study agreed with the hypothesis that there are regional differences of the MTS. Primary second molar occlusal dentin showed regional variations in tensile strength with the highest strengths in the central area. Regional differences of the MTS of dentin might influence to the prognosis of dental restoration. Cavity design should take biomechanical properties of dentin to consideration. Future study is required to decide the suitable cavity design.

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