Anisotropic ultimate strength and microscopic fracture patterns during tensile testing in the dentin–enamel junction region

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The dentin–enamel junction (DEJ), which is the interface between the outer tooth enamel and the underlying dentin, has unique biomechanical properties that allow the two different materials to function together without fracture. The aim of this study was to investigate the anisotropic ultimate tensile strength of the DEJ. Twenty dumbbell-shaped samples were prepared from 10 extracted human molar teeth for tensile strength testing. The maximum tensile forces at fracture were measured in the direction parallel (10 samples) and perpendicular (10 samples) to the DEJ. The mean tensile strength of the samples was significantly higher under parallel forces (30.9±3.3 MPa) than under perpendicular forces (20.6±4.8 MPa; $p<0.05$). Our findings confirm the structural anisotropy of the DEJ.

Keywords: Tooth, Dentin–enamel junction, Dentin, Enamel, Tensile strength

INTRODUCTION

The mechanical properties of teeth provide useful information in clinical dentistry. Teeth are constituted of a crown and a root or roots as shown in Fig. 1A. In both parts, dentin is the most abundant mineralized tissue by both weight and volume (Fig. 1B). Additionally, dentin is covered by enamel as shown in Fig. 1B. The dentin–enamel junction (DEJ) is the interface between the enamel and dentin (Fig. 1B) and is designed to allow these two different materials to work together without fracture during mastication. The DEJ may play an important role in inhibiting catastrophic tooth fracture.

The mechanical properties of the DEJ were first recognized from micro-hardness profiles; enamel and dentin near the DEJ recorded the lowest hardness values[1]. The strain measured across this zone when a compressive load was applied longitudinally to the tooth axis was shown to decrease from the DEJ towards the central dentin[2]. Rasmussen found it is difficult to induce tooth fracture at the DEJ[3]. Pioch and Staehle measured the shear strength of the DEJ, but fracture areas were mainly in the dentin and never exactly at the DEJ[4]. Lin *et al.* investigated cracks in the enamel, and found that the cracks ran past the DEJ and into the dentin, but did not run perpendicular to the interface[5]. Fracture toughness has also been investigated[6], and several investigations of the DEJ have measured hardness and fracture toughness with microindentation and nanoindentation tests[7-9].

However, little information is available about the ultimate tensile strength (UTS) of the DEJ, and previous studies have examined the UTS of the DEJ in only one direction[10,11]. Tensile tests have reported useful methods for easily identifying defects; and it has been found that structural features caused the failure[12-14]. It is possible to identify structurally weak regions in the DEJ using tensile tests. Information is also limited regarding the anisotropic UTS of the DEJ. No studies have analyzed the anisotropic strength at the DEJ. It is known that enamel and dentin are highly anisotropic structures[15-19]. It is rare to see enamel that does not separate from dentin, and chipping of enamel is common in mammals in general[20,21]. Thus, it is important to identify the anisotropic UTS of the DEJ to understand the structure of the DEJ. Hence, the purpose of this study was to investigate the anisotropic UTS of the DEJ structure and to observe microscopic fracture patterns during tensile testing in the DEJ.

MATERIALS AND METHODS

Materials
Ten extracted, caries-free human third molars were obtained from patients aged 26–35 years, with informed consent. The molars were extracted because of pericoronitis. The teeth were stored in Hank's balanced saline solution (HBSS) at 4°C, and were used within 1 month of their extraction according to previous reports[12-14]. The protocol of this study was approved by the Ethics Committee of Showa University School of Dentistry.

Preparation of specimens
The 10 molars were sectioned midway along the mesiodistal plane along the long axis of the tooth using a diamond saw (Isomet, Beuhler, Lake Bluff, IL, USA). Two dentin slabs, approximately 1 mm thick, were cut from each tooth (Fig. 1C). A rectangular block (3.0×3.0×1.0 mm) was harvested from each dentin slab (Fig. 1D). Then, dumbbell-shaped specimens (central portion; 1.0×1.0×1.5 mm, Fig. 1E) from each rectangular block were prepared using a profiling machine. Dumbbell-shaped specimens were useful for identifying defects easily[12-14,19]. Further, we employed
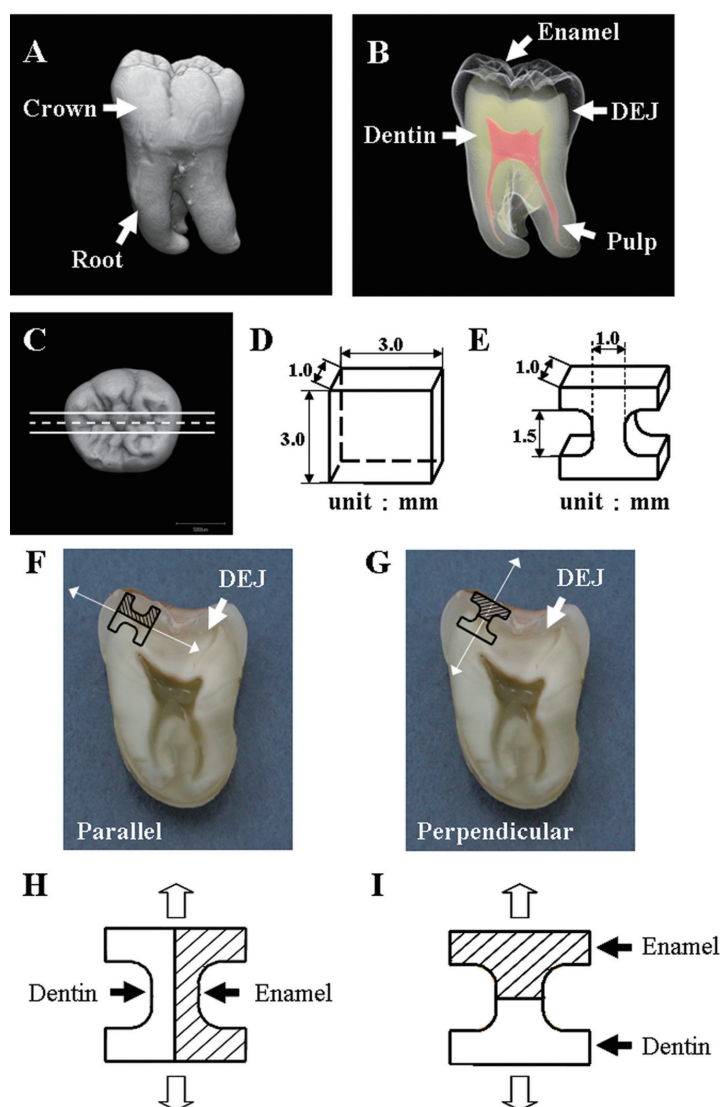
Fig. 1 Illustrations of the tooth (A) outer structure and (B) inner structure. (C) The location of dentin slabs in human molar tooth. Schematic representations of (D) rectangular block and (E) dumbbell-shaped specimen. The location of the tensile test specimen from a human molar tooth slab: (F) PA (subjected to tensile loading parallel to the DEJ); and (G) PE (subjected to tensile loading perpendicular to the DEJ), where arrows show the tensile test orientation. Schematic diagrams of dumbbell-shaped specimens: (H) PA; right side is enamel, and left side is dentin, and (I) PE; upper side is enamel, and lower side is dentin, where arrows indicate the tensile orientation.

miniaturized dumbbell-shaped specimens for tensile testing in previous study[12-14,19]. The specimen size and shape affected tensile strength. Thus, these same size and shape are selected to use for this tensile study.

Specimens were prepared in the enamel, DEJ and dentin of the external slope in the occlusal area for two experimental groups: PA specimens were subjected to tensile loading parallel to the DEJ (Fig. 1F); and PE specimens were subjected to tensile loading perpendicular to the DEJ (Fig. 1G). In other words, in PA specimens, the right side is enamel, and the left side is dentin (Fig. 1H); and in PE specimens, the upper side is enamel, and the lower side is dentin (Fig. 1I). Additionally, specimens were stored in HBSS to retain moisture[12-14].

Tensile strength test
The specimens were set on a universal testing machine (EKO Instruments, Tokyo, Japan) immediately after preparation. The specimens were stored in 37±0.5°C HBSS during the tensile strength test. The maximum load was used to calculate the strength. Ten specimens in each experimental group (PA and PE) were tested, and the mean tensile strength of the specimens was calculated in each group. The results were analyzed using ANOVA and Tukey's multiple comparison test (α=0.05).

Observations of the fracture surface

The fractured specimens from the tensile strength tests were fixed and coated according to previous reports[12-14]. The fractured surfaces were then observed using a scanning electron microscope (S-4700, Hitachi, Tokyo, Japan).

RESULTS

Tensile strength of the DEJ region

The tensile strengths of the two groups are shown in Fig. 2. The mean tensile strengths were 30.9±3.3 MPa (PA) and 20.6±4.8 MPa (PE). The tensile strength of the parallel junctions was significantly higher than that of the perpendicular junctions ($p<0.05$). Figure 3 shows a schematic diagram of the fracture specimens after tensile strength testing.
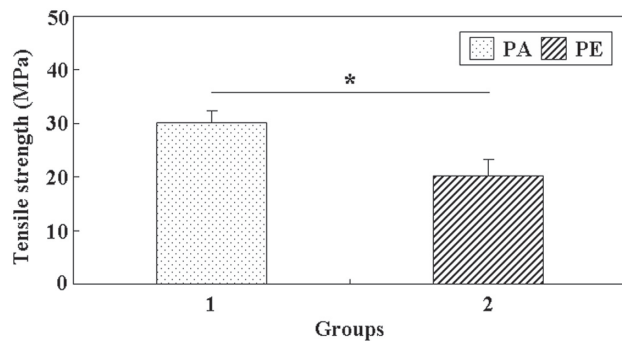

Fig. 2   Tensile strength of the two groups. An asterisk indicates statistical significance ($p<0.05$).

SEM observations of enamel, dentin and the DEJ

The fractured surface is shown at low magnification in Fig. 4. In the PE group, the fracture occurred above the DEJ; thus, only enamel is visible in the fractured surface, not the DEJ or dentin (Fig. 4B). At higher magnification, the DEJ can be seen to arrest crack propagation (Fig. 5A), and SEM images show the fibrous matrix with the collagen fibers and mantle dentin (Fig. 5B). Mantle
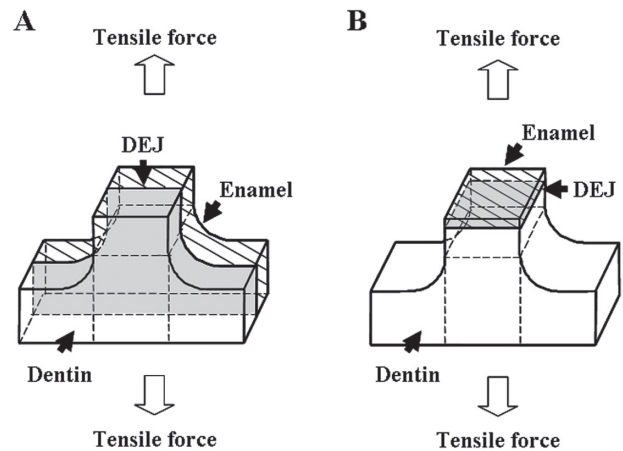

Fig. 3   Schematic diagram of fracture specimens after tensile strength testing: (A) PA; and (B) PE.
In specimens subjected to a parallel load (PA group), the fracturing occurred half in the enamel and half in the dentin (A). In specimens exposed to a perpendicular load (PE group), all fracturing occurred above the DEJ within the enamel and neighboring the DEJ (B).
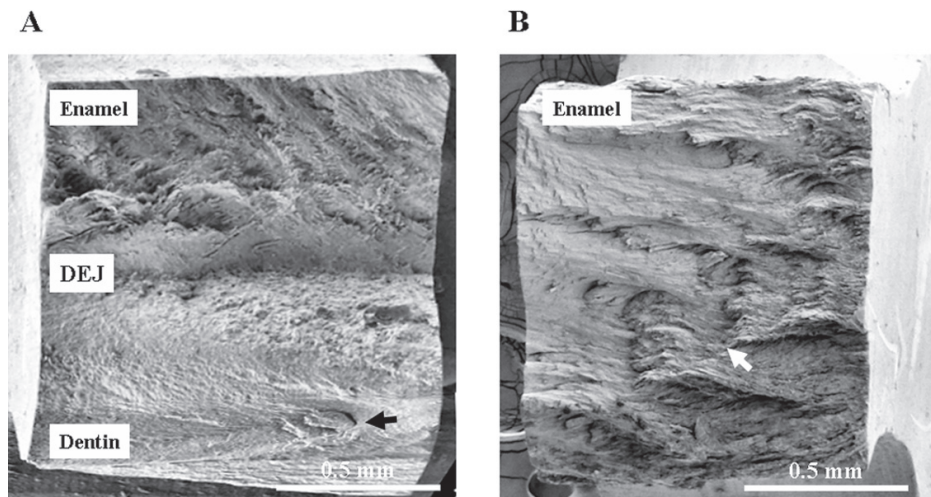

Fig. 4   Fractured surface representation after tensile strength testing at low magnification: (A) PA; and (B) PE.
The origin of the fracture in the PA group is indicated by a black arrow (A). Crack propagation clearly ran from the origin, and cracks can be seen near the inner dentin. Cracks were observed within the dentin, but they have been prevented from running into the DEJ. However, cracks in the inner enamel were also observed. The inner and outer enamel are distinguishable from each other. The inner and outer enamel differed in the orientation of their crack propagation. In the PE group (B), the fracture origin is indicated with a white arrow.

dentin is the outer layer of dentin that is different from the bulk of dentin. Figure 6 shows SEM micrographs of the fractured surfaces of PA specimens. Typical fractures of the enamel are shown in Figure 6A (inner enamel) and 6B (outer enamel). The white arrows indicate cracks, which pass through the change in orientation from inner to outer enamel; crack propagation can be seen running at an angle of approximately 45° to the DEJ and being deflected by 90° in the transitional region between the inner and outer enamel. Fractures occur preferentially and transversely to the enamel prisms.

Prism fractures then occur obliquely and leave prisms in the inner enamel (arrows, Fig. 6A). However, in the outer enamel of the fractured surface of the specimens, fractures occur preferentially along the enamel prisms (arrows, Fig. 6B). The prisms are cleaved, and porosity is seen in the surface of the fractured prisms (Fig. 6C). Additionally, high magnification SEM micrographs of enamel prisms show the hydroxyapatite (HAP) crystals both in the prism and interprisms (Fig. 6D). The prism shows parallel HAP crystallites, and the interprism also shows perpendicular HAP crystallites (arrows).
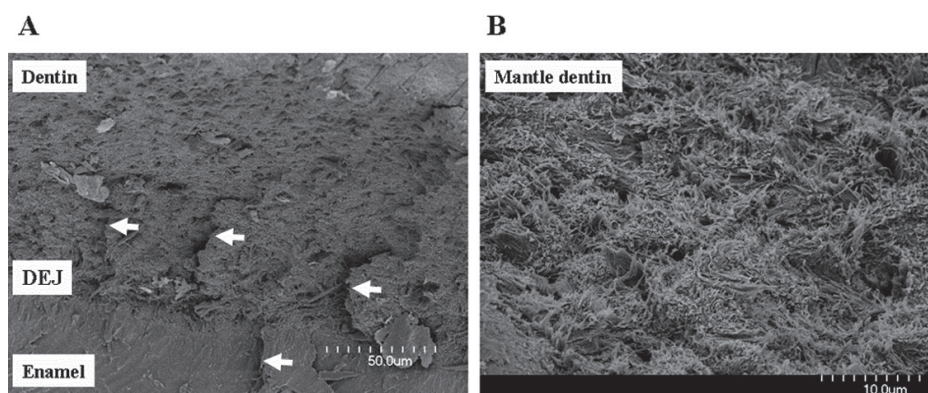

Fig. 5   Fractured surface representation after tensile strength testing of PA: (A) DEJ region, and (B) mantle dentin.
At this higher magnification, cracks can be seen running to the DEJ both in the enamel and dentin regions as indicated by the arrows. The DEJ can be seen to arrest crack propagation in the dentin and prevent failure, and the cracks in the enamel also arrest crack propagation and prevent failure (A). SEM images of the fractured surface show the presence near the DEJ of porous fibrous reticulate intertubular dentin with collagen fibers (B).
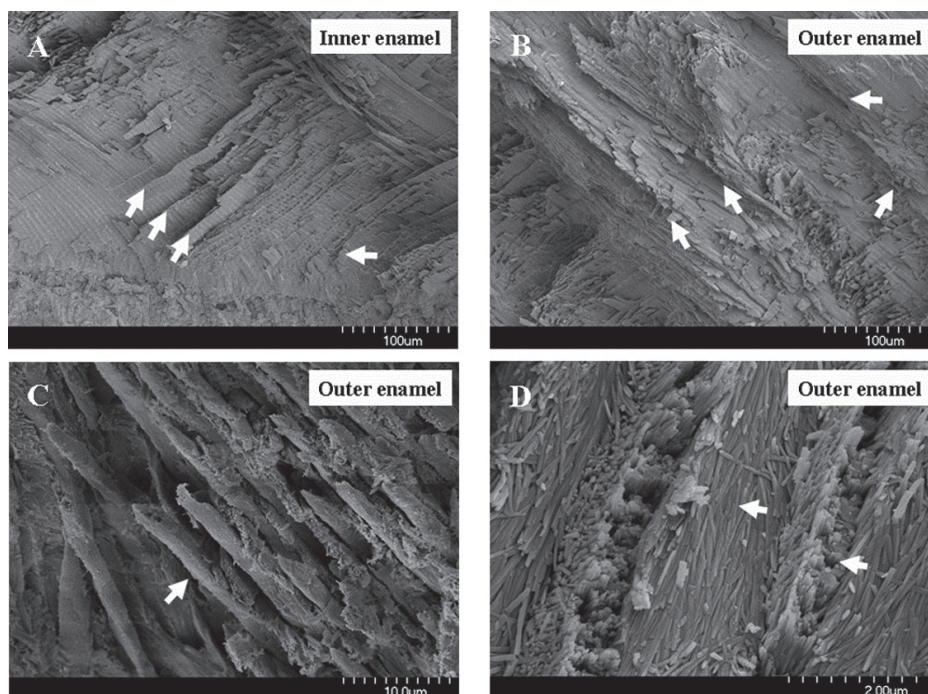

Fig. 6   Fractured surface representation after tensile strength testing of PA: (A) lower magnification of the inner enamel, (B) lower magnification of the outer enamel, (C, D) higher magnification of the outer enamel.

DISCUSSION

The results of this study confirm the presence of structural anisotropy in the DEJ. The DEJ is anisotropic, and its strength is considerably lower when stressed perpendicular to the orientation of the DEJ.

The tensile strength of the PE group was approximately 20 MPa. Previous studies on the tensile strength of tooth structure along the axis of the tooth have reported that the strength of enamel is 20 MPa[17], and that of dentin is 40 MPa[14]. Another study examined the UTS using a microtensile technique[11] and recorded mean UTS values of 47 MPa (parallel to the DEJ); 42 MPa (parallel to the enamel); 11 MPa (perpendicular to the enamel); and 62 MPa (superficial dentin). They also found that the UTS of the DEJ is closer to enamel than to dentin, which is in agreement with our results. However, SEM observations revealed that the fracturing occurred within the enamel, and never in the DEJ. Reasons for the lack of a pure interfacial failure of the DEJ are that the DEJ is stronger than the enamel, and that the DEJ structure is complex and is able to modify crack propagation.

Several studies have examined the tensile strength of the DEJ. In a study in which specimens were prepared from the buccal surface at its greatest point of contour, all specimens fractured near the DEJ[10]. Three types of fracture pattern were observed: 50% of specimens fractured in the superficial dentin; 10% fractured in the enamel; and 40% fractured in the DEJ area[10]. These fracture patterns differed from those in our study. Fractures never occurred at the interface between the enamel and dentin. Our specimens were prepared from the occlusal area of molars, and all occlusal DEJ specimens fractured within the enamel. As mentioned above, the position of the sampled specimen (for example occlusal or cervical) may affect the properties of the tested materials. Examination of the DEJ surface has shown that both enamel and dentin are scalloped in nature[22,23]. However, the scallop size and shape vary with tooth type; the scallops of posterior teeth are larger than those of anterior teeth[24]. Whittaker reported that the DEJ was more scalloped in the occlusal portion of the molars, became less scalloped in the buccal region, and was almost flat and relatively non-scalloped in the cervical region[25]. Another study found that the width of the DEJ was 13 μm in the occlusal region and 6 μm at the cervical region; the difference in the width of the DEJ was confirmed in these positions[26]. Additionally, the DEJ zone is composed of different organic and inorganic components at the occlusal and cervical positions[26]. It has been suggested that the DEJ scallops provide increased surface area and thus reduce interfacial stress concentration[27]. Therefore, structural differences between the occlusal and cervical DEJ specimens would have resulted in different fracture patterns. The differences might also be related to differences in function, with the occlusal surfaces exposed to greater loads during mastication than the cervical area[26].

This study confirmed that the enamel above the DEJ is weaker than the DEJ and the dentin below the DEJ. In clinical dentistry, enamel cracks are observed relatively often in sound teeth[28]. Additionally, chipping of the enamel is common in general but it is rare to see chipping in the DEJ; that is, the enamel does not separate from the dentin at the DEJ[20,21]. These findings are consistent with our study. Specialized, highly mineralized first-formed enamel is present close to the DEJ[29,30]. The fracture toughness of the DEJ zone was investigated using Vickers microindentation in a previous study, which found that the crack deflection primarily occurs in the first-formed enamel[7]. This first-formed enamel was a zone of peak hardness and radiodensity[31]. The first-formed enamel may act as a shield helping to avoid catastrophic interfacial failure[7].

The tensile strength of the PA group was approximately 30 MPa. The DEJ was well bonded with the enamel and dentin, and there was no fracture at the DEJ. The surface fractures occurred from the inner dentin. The inner dentin tubules might serve as sites for crack initiation[32]. Previous studies on the tensile strength of tooth structure have reported that the strength of dentin is 60 MPa[14] when tested with the load applied transversely to the orientation of the dentinal tubules. We tested the same specimen size as in a previous study (approximately 1.0 mm^2). Thus, the strength of the dentin part was approximately 30 MPa because it was half the size (approximately 0.5 mm^2). Additionally, enamel tensile strength is lower than that of dentin[11], so when a specimen is subjected to tensile loading perpendicular to the DEJ, the tensile strength could be dominated by the dentin (30 MPa), resulting in a theoretical strength of the DEJ of 30 MPa. Our data indicated a value of approximately 31 MPa, in agreement with the experimental data.

Additionally, SEM images show a porous reticulate matrix of dentin near the DEJ –the mantle dentin. This zone is also thought to play an important role, functioning as a cushion or gasket that allows the enamel and dentin to work together[33].

Enamel consists of outer enamel and inner enamel. Outer enamel is close to the tooth surface with the long axes of the enamel rods being straight and parallel. Inner enamel is close to the DEJ where the enamel rods are interwoven or decussated with a smooth transition between both enamel types[34]. In this study, crack propagation occurred and ran at an angle of approximately 45° to the DEJ in the inner enamel, and the cracks were deflected by 90° in the transitional region between the inner and outer enamel. Three-point bending experiments were performed in a previous study[35]. In agreement with our study, the results revealed that within the enamel layer, cracks propagated at 45° to the sample surface and were deflected by 90° in the inner and outer enamel. In the inner enamel, oblique crack propagation was not caused by the orientation of the enamel rods. They found that in the inner enamel layer, rods no longer determine the crack paths because they are decussated. Furthermore, the structure from outer to inner enamel increased in toughness[36]. Decussated

enamel plays an important role in the direction of crack extension[37]. Therefore, the crack resistance changes from outer to inner enamel. Thus, in the lifetime of a tooth, the enamel transitions are also shielded from tooth failure.

CONCLUSIONS

The mechanical properties of tooth structure are important for understanding tooth fractures. Failures did not occur in the DEJ subjected to tensile loading perpendicular to the DEJ. The enamel above the DEJ is weaker than the DEJ and the dentin below the DEJ.

ACKNOWLEDGMENTS

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