journal

J. Am. Ceram. Soc., **83** [1] 238–40 (2000)

The Dentino-enamel Junction is a Broad Transitional Zone Uniting Dissimilar Bioceramic Composites

Shane N. White,*,†,‡ Michael L. Paine,† Wen Luo,† Mehmet Sarikaya,§ Hanson Fong,§ Zhaokun Yu,† Zhen C. Li,† and Malcolm L. Snead†

Center for Craniofacial Molecular Biology, School of Dentistry, University of Southern California (USC), Los Angeles, California 90033, School of Dentistry, University of California at Los Angeles (UCLA), Los Angeles, California 90095, and School of Materials Science and Engineering, University of Washington, Seattle, Washington 98195

The dentino-enamel junction (DEJ) couples a hard, brittle, stiff layer of enamel to underlying flexible and resilient dentin. Prior microscopic images have shown an abrupt demarcation between enamel and dentin; however, new data on protein expression suggests a graded transition. In this study, Vickers microindentations reveal a gradation in hardness with a wide transitional zone across the DEJ region. Therefore, the DEJ must be regarded as a broad transitional region between two interacting tissues, not as a discrete interface. This zone may reduce stress concentrations and act as an important toughening mechanism between mechanically and structurally dissimilar tissues.

I. Introduction

Teeth are remarkably successful, natural, bioceramic composite structures. Teeth are composed of two bioceramic composites: a hard outer layer of enamel that resists wear and acid attack and dentin, which is a flexible, resilient internal layer that provides fracture resistance. Enamel is a stiff, brittle ceramic composite that is composed of a complex meshwork of hydroxyapatite (HAp) prisms with a small amount of organic material between the rods; each prism is, in turn, made of many small individual HAp crystals. Dentin is a strong, tough, flexible composite ceramic that forms the bulk of the tooth and consists of a mineralized protein-rich matrix that forms around a series of tubules. Interfaces between such dissimilar materials usually concentrate stresses and delaminate. However, the dentino-enamel junction (DEJ) is remarkably successful in transferring applied loads from enamel to dentin.[1,2] Despite a human lifetime of repeated masticatory, parafunctional, and occasional impact loading, enamel rarely separates from dentin. Furthermore, the DEJ is highly damage tolerant. Cracks that initiate in enamel often are deflected or limited by the DEJ. Hence, most cracks do not continue to propagate into dentin and through the entire tooth.

It has been proposed that the crack-resistant properties of the DEJ may originate from a gradation of mechanical properties, rather than an abrupt transition,[1,3] among other mechanisms.[4–8] Rather than a gradation in structure between the enamel and the dentin, light microscopy and scanning electron micrography (SEM) have suggested an abrupt demarcation between enamel and dentin (Fig. 1).[6–8] Such studies show a narrow but distinct interface between the enamel and the dentin. This interface is thought to represent the original position of the basement membrane of the ameloblasts and odontoblasts, where they contact in the embryological tooth bud. When biomineralization starts, the ameloblasts and odontoblasts move away from each other in opposite directions, leaving protein-rich matrices in their respective wakes. These protein-rich matrices guide mineralization. Thus, this optically distinct line is usually thought to represent an abrupt demarcation between these matrices.

Teeth develop as a consequence of reciprocal and sequential interactions between tissues of dissimilar germ-layer origins. Ectoderm-derived ameloblasts and ectomesenchyme-derived odontoblasts exchange a multitude of signals that are initiated by the oral ectoderm.[9] Until recently, the expression of matrix proteins was believed to be germ-layer specific; i.e., ameloblasts or odontoblasts only expressed proteins that were exclusive to themselves. On the contrary, dentin sialophosphoprotein (DSPP) has

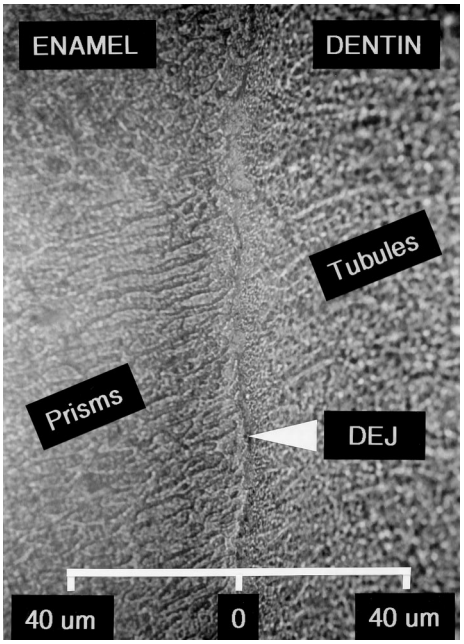

Fig. 1. Reflected-light micrograph of the DEJ; a distinct boundary between enamel and dentin is visible. Enamel prisms can be observed on the enamel side, but the more widely spaced dentin tubules cannot be observed clearly on the dentin side, because their open lumens fall beneath the focal plane.

K. J. Anusavice—contributing editor

Manuscript No. 189433. Received April 8, 1999; approved September 21, 1999.
Supported by the National Institutes of Health, National Institute of Dental and Craniofacial Research, under Grant No. DE12420, DE06988, and DE11704.
*Member, American Ceramic Society.
†Center for Craniofacial Molecular Biology, School of Dentistry, USC.
‡School of Dentistry, UCLA.
§School of Materials Science and Engineering, University of Washington.

been shown to be briefly expressed by ameloblasts during the formation of the earliest enamel; however, soon thereafter, DSPP is only expressed by odontoblasts.[10–13] Similarly, ameloblastin (Ambn) is transiently expressed by odontoblasts during initial enamel formation; however, its expression is quickly restricted to ameloblasts.[14,15] Amelogenin, which is a protein that is expressed only by ameloblasts, is translocated to odontoblasts during early enamel formation.[16] This pattern of protein expression may represent an exchange of signals of dissimilar germ layers that contribute to the formation of the DEJ and provide a pathway to a gradation in mechanical properties. This unique mix of proteins, which exists only during DEJ formation, may define the unique properties of the DEJ.

We have performed hardness testing of human teeth, to ascertain if they have a gradation in mechanical properties at or near the DEJ. This gradation would be reflective of organized protein mixing during tooth development.

II. Experimental Procedure

Ten freshly extracted human incisor teeth, which were kept moist at all times during extraction, mounting, polishing, and testing, were embedded in slow-set epoxy resin and sequentially ground to a 0.1 μm alumina finish using a semiautomatic polisher (Buehler, Lake Bluff, IL). The teeth were ground longitudinally in the sagittal plane and refinished immediately before testing.

A custom-made Vickers microhardness testing machine (Mark V, East Granby, CT) was used to make indentations in the ground and polished specimens. The Vickers microhardness scale is useful because it extends beyond the microhardness range, up to the macrohardness range, using the same indenter. Thus, a very wide range of loads can be used. Hardness is a useful descriptive parameter, because it provides an approximate quantitative measure of enamel mineralization and dentin density.[17] Mature enamel normally contains ~97 wt% inorganic HAp, whereas dentin normally contains open tubules and only ~75 wt% HAp. Thus, the hardness values differ considerably between enamel and dentin. In this study, relative differences in hardness were used to describe the transition from enamel to dentin across the DEJ region.

The perpendicular distance from the center of each indentation to the DEJ was measured using a video micrometer (Javelin, Torrance, CA). The DEJ was defined as the optically visible dark line that separates enamel and dentin. More indentations were made near the area of primary interest—the DEJ—than in peripheral areas of the profiles. The indentations were clustered together in the same area of each tooth but were spread at least 3 times their diagonal lengths apart from each other.

Hardness profiles were made perpendicularly across the DEJ in the incisal third of the facial region of each tooth. Initially, a hardness profile was composed, using a load of 15 g with thirty indentations per tooth over a total profile length of 400 μm (n = 300). A second series of indentations was made in the DEJ area using a load of 500 g with twenty indentations per tooth (n = 200). Because of the large footprint that was produced by loads of 500 g, these indentations were intended to confirm bulk hardnesses rather than produce detailed profiles. Scatter plots were drawn of the pooled data, and a curve was superimposed on the 15 g data. The curve was drawn by calculating mean values for pooled data points every 25 μm within the bulk enamel and dentin, as well as every 7.5 μm within 60 μm of the optical DEJ. Then, these mean values were connected to form the curve.

III. Results

The 15 g microhardness plot is illustrated in Fig. 2. This plot shows a broad gradation in hardness across the DEJ region. Both enamel and dentin that are adjacent to the DEJ contribute to this gradient. In addition, a small peak in hardness is observed ~10 μm on the enamel side of the DEJ, and a small dip is visible on the dentin side of the DEJ.

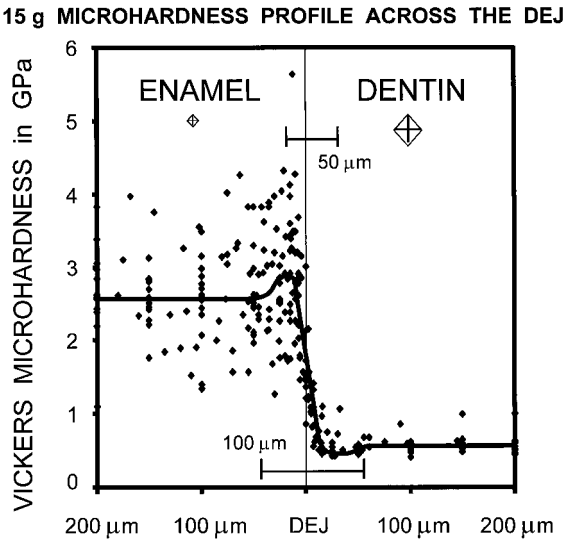

Fig. 2. Vickers microhardness profile across the DEJ with loads of 15 g. Indentation footprints are drawn to scale in the top portion of the figure, and a distinct gradation in hardness is visible through the DEJ area. Bars indicate that the broad transition zone from bulk enamel to bulk dentin is ~100 μm wide, and that the distance from the enamel peak to the dentin dip is ~50 μm. Linear portion of the transition is ~27 μm wide.

This 500 g hardness plot confirmed that the hardness values of enamel far away from the DEJ, dentin far away from the DEJ, and the hardness of the DEJ itself were similar to those produced by the 15 g indentations (Fig. 3). Because the 500 g indentations had extremely large footprints, the appearance of a more-gradual transition in Fig. 3 was produced primarily by averaging the dentin and enamel hardnesses within individual indentations close to the DEJ.

IV. Discussion

The biomechanical transitional zone between bulk enamel and bulk dentin, as indicated by the bars in Fig. 2, is ~100 μm wide. This transitional zone is large, relative to the optical DEJ, which seemed to be <2 μm wide. The optical DEJ defines the apparent

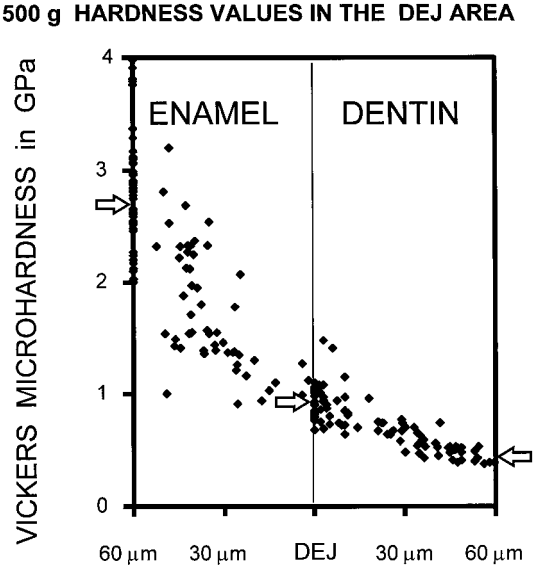

Fig. 3. Vickers hardness values in the DEJ area with loads of 500 g; the extremely large indentation footprints (40–160 μm) limit this data to confirmation of bulk enamel, DEJ, and bulk dentin hardness values (indicated by arrows).

identity of enamel or dentin and suggests a clear, abrupt demarcation between them. In contrast, the biomechanical transition zone is broad and reflects the functional union between enamel and dentin. This biomechanical transitional zone can reduce stress concentrations at the interface between enamel and dentin profoundly.

Our results indicate that very early enamel and dentin—the so-called "aprismatic enamel" and "mantle dentin"—close to the DEJ differ from bulk enamel and dentin. If so, this result must have a genetic basis. As with all biological tissues, the development of structural mineralized tissues such as enamel and dentin is controlled by specific genes and the proteins they encode. Because the cells produce protein-rich matrices that govern mineralization, any local changes in enamel or dentin must have a genetic origin and are produced by changes in protein expression at specific developmental periods. Each cell that forms enamel or dentin exerts a field of influence only 2–8 μm in diameter parallel to the DEJ; however, the fields stretch up to several millimeters in length perpendicularly away from the DEJ. The biomechanical properties of this field of influence must reflect programmed changes in gene expression. The existence of aprismatic enamel is additional evidence of changes in gene expression during the secretory phases of ameloblasts.[18,19]

A small microhardness peak located ~15 μm from the DEJ was observed (Fig. 2). This small peak was unexpected and could have been simply a manifestation of the high intrinsic variability of enamel and the sample size. However, because this was the area of interest, proportionately more indentations were made there, but the peak in hardness persisted. When separate profiles were plotted for the ten individual teeth, six plots showed this peak, two plots showed no peak, and two plots showed a slight dip at this location. Variability is a hallmark of complex biological systems.

In contrast to the hardness peak that was located 15 μm from the DEJ, the enamel even closer to the DEJ was softer than bulk enamel. This zone, which is very close to the DEJ, may contain more protein than other types of enamel or other types of protein that is not found in bulk enamel. Such protein could originate from several sources. Collagen, a structural protein, is produced by odontoblasts and extends into enamel.[1,5] Ameloblasts may express different types of protein, such as DSPP, during the initial stages of enamel formation.[10–13] In bulk enamel, most of the protein produced by ameloblasts is broken down and removed during subsequent mineralization.[20–23] Changes in breakdown and removal also could allow selective retention of protein close to the DEJ. Finally, a reciprocal exchange of protein between the enamel and the dentin also could quantitatively and qualitatively alter protein levels in enamel very close to the DEJ.

Tissue on both sides of the DEJ contributes to the gradation in hardness, which suggests that localized differences can be found in dentin as well as in enamel. The gradation in hardness in dentin close to the DEJ could be related to the division, branching, and narrowing of the hollow dentinal tubules as they approach the DEJ.[24]

The microhardness profile across the DEJ (Fig. 2) was consistent with a microradiodensity profile across the DEJ that was previously published by Elliott *et al.*[3] Common features to both curves include a graded transition from enamel to dentin, a small peak on the enamel side of the DEJ, and a dip on the dentin side of the DEJ. Wang and Weiner[25] related a decrease in microhardness on the dentin side of the optical DEJ to an area of greatly increased strain and concluded that dentin contains localized structural adaptations for transferring and reducing stress.

More work is needed to reconcile the abrupt discontinuity that is observed in light microscopy and SEM investigations in the broad transitional zone found in this current investigation and suggested by recent data on protein expression. An analysis of biological structures is difficult because of intrinsic variability, the small structural scale, and a composition of complex mixtures of similar proteins.

The long-term goal of this work is to use biomimetic principles to design an artificial DEJ, which is important for the repair of teeth that have been damaged by disease or trauma. Many artificial substitutes for enamel and dentin exist; however, their permanent attachment to and mechanical integration with damaged teeth remains a formidable problem. Such junctions also can be used to design artificial or replacement skeletal joints.

V. Conclusion

The results of this experiment show that the dentino-enamel junction must be regarded as a broad transitional region that is a reflection of enamel and dentin protein expression, not as an abrupt interface. A gradation in physical properties across this zone may be an important toughening mechanism that reduces stress concentrations between mechanically dissimilar enamel and dentin.

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