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The dentin–enamel junction—a natural, multilevel interface

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Abstract

Teeth contain two major calcified tissues, enamel and dentin, that are joined by an interface known as the dentin–enamel junction (DEJ). Enamel is the hard and brittle outer portion of the tooth that cuts and grinds food and dentin is composed of a tougher biological composite, that can absorb and distribute stresses. The DEJ is a complex and critical structure uniting these two dissimilar calcified tissues and acts to prevent the propagation of cracks from enamel into dentin. The DEJ has a three-level structure, 25–100 μm scallops with their convexities directed toward the dentin and concavities toward the enamel; 2–5 μm microscallops; and a smaller scale structure. Mechanical properties measurements, chemical differences and imaging have been used to determine the functional width of the DEJ. AFM based nanoindentation gave values of 11.8 μm, microRaman yielded a width of 7.0 μm, while the smaller probe used for AFM nanoscratching yielded 2.0 μm, and values from dynamic modulus mapping were less than 1 μm. The unique architecture of the DEJ may account for this variation based on enamel–dentin phase intermixing. The ultimate goal is to use the DEJ as a biomimetic model for other interfaces joining dissimilar materials.

Keywords: Biomedical applications; Hardness; Interfaces; Mechanical properties

1. Introduction

The dentin–enamel junction (DEJ) appears to have unique qualities that permit the joining of highly dissimilar calcified tissues in teeth. Fig. 1a shows an extracted tooth, and a sagittal section through the center of the tooth (Fig. 1b) that identifies the enamel, dentin and the DEJ. Despite considerable study,[1–9] many questions remain concerning the characteristics of the junction, including: variations in its multi-level scalloped structure, its mechanical properties, its functional width, whether it has a unique composition, and its fracture properties that may suggest the mechanisms by which it acts to retard crack propagation. Answers to these questions should provide tools needed to use a biomimetic approach to joining dissimilar materials. Advances in imaging methods and AFM-based nanoindentation now provide methods that should provide the information needed to implement such a biomimetic approach. This paper reports on recent studies that provide some of this important information.

1.1. Microstructure of enamel, dentin and the DEJ

The dentin–enamel junction (DEJ) unites two dissimilar calcified tissues. Enamel is the hard and brittle outer portion of the tooth and is mainly comprised of a defective carbonate rich apatite arranged in enamel rods or prisms 4–5 μm in diameter that lie nearly perpendicular to the DEJ and which contain highly oriented and very long crystals of apatite.[10,11] In contrast, dentin is composed of a tougher biological composite, similar in many respects to bone, with a unique architecture consisting of dentinal tubules approximately 1 μm diameter containing odontoblastic cell processes that are surrounded by peritubular dentin, cylinders of approximately 0.5–1 μm thickness of randomly oriented apatite crystallites[12] (See Ref. 13 for review). These tubular units are embedded in a collagen matrix-apatite reinforced composite. Since the tubules are the formative tracks of the odontoblastic cells that move inward and reside on the pulp chamber surface, of smaller coronal area than the DEJ, there are substantial variations in morphology and structure of the dentin from the DEJ to the pulp chamber or pulp–dentin junction (PDJ).

The DEJ is a complex and critical structure uniting these calcified tissues. It plays critical roles as the

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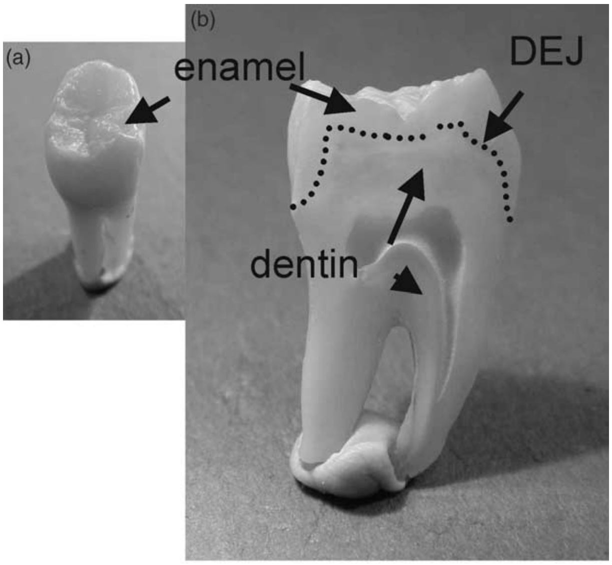

Fig. 1. Photograph of an extracted human tooth (a) showing enamel on the outer surface and a sagittal section (b), showing enamel, dentin, and the DEJ.

initiation surface for ameloblastic and odontoblastic activity during tooth formation,[10] and in maturity is probably critical to the biomechanical integrity of the tooth.[5] Cracks propagate readily through the enamel, but generally do not cross the DEJ (Fig. 2). This paper is most concerned with the characteristics of the DEJ in the mature tooth as it is related to its biomechanical structure–function relationships. It is widely held that the function of the DEJ is the joining of these structurally diverse calcified tissues. Thus it unites enamel, the hardest and most brittle of the calcified tissues, geared to cutting and grinding with minimal wear, to a substantially tougher biological composite that can absorb and distribute stresses. The structure of the DEJ is generally described as scalloped with its convexities directed toward the dentin and concavities directed toward the enamel as seen in Fig. 3.[11] This adaptation is thought to lead to enhanced bonding between these calcified tissues.

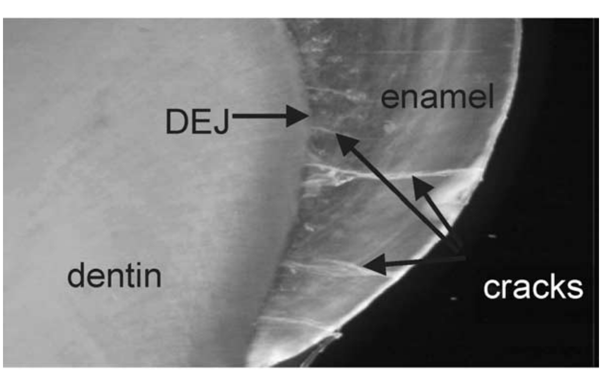

Fig. 2. Optical micrograph of a cross-section through a tooth, showing cracks that have propagated through the enamel, but stop at the DEJ, without penetrating into the dentin.

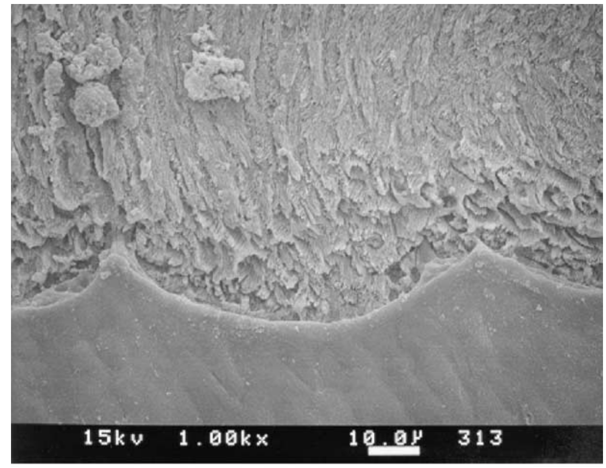

Fig. 3. SEM micrograph showing scallop structure in DEJ, with enamel on the top and dentin on the bottom.

However, there is little information available on the size and size variations of these structures, and there may be considerable variability between animals, individual teeth and within a given tooth.[9,14] Whittaker[9] examined 162 deciduous and permanent teeth from humans and monkeys and found considerable variability. In human teeth the scallop size varied considerably and frequently appeared to range from 25 to 100 μm. Each scallop appears to have a substantial range of microstructure. Interestingly, it was found that proximal surfaces were more scalloped than buccal or lingual surfaces;[9] although Scott and Symons[14] reported more scalloping near the cusps, and Schour[15] suggested there was more scalloping in the gingival third.

Lin et al.[4] studied the DEJ using high resolution SEM and immuno-labelling to identify collagen. They found that the scalloped structure contained microscallops as well as collagen type I fibrils that appeared to emanate from the dentin and coalesce to form fibrils approximately 100 nm in diameter that crossed the DEJ and inserted into the enamel mineral. Variation in shape and size of the scallops was reported, with typical values appearing to be 25–40 μm from the published images, and they reported that each scallop appeared to contain a finer sequence of microscallops. In addition, the available published micrographs show finer structures that have not as yet been defined. It appears that the DEJ forms a complex interface with at least three levels of microstructure: scallops of varying size that may vary with location; microscallops housed within each scallop; and a finer nanolevel structure within each microscallop. For convenience we will call this the three level microstructure model of the DEJ or 3LM model. An AFM image of scallops, microscallops and the finer structure is shown in Fig. 4a, and collagen fibrils, identified by their characteristic 67 nm banding pattern, are shown in Fig. 4b.

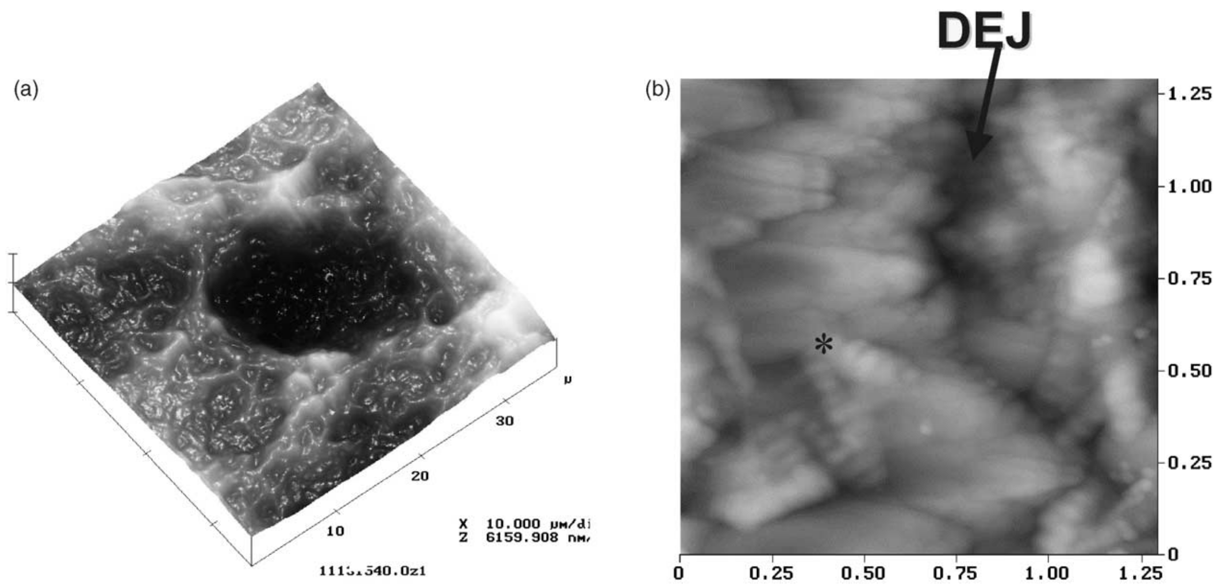

Fig. 4. AFM images showing (a) the three-dimensional scallop structure and (b) the penetration of collagen fibrils across the DEJ. Examples of collagen fibrils are labeled with an *.

It is well known that the DEJ is less mineralized than either enamel or bulk dentin, contains a higher organic matrix, and is probably associated with the first formed mantle dentin.[4,10] As pointed out by Lin et al.[4] the DEJ may be a microstructurally distinct and mechanically tougher portion of the tooth that is essential to its function and acts to prevent enamel cracks from propagating across the interface, thus preventing catastrophic tooth fractures. It is of interest to note that on clinical examination, almost all people have a substantial number of deeply penetrating cracks in the enamel of their teeth (Fig. 2). Such flaws would be expected to propagate and lead to early, catastrophic failures of tooth structure. In spite of this basic biomechanical principle, tooth fracture is uncommon, especially if the teeth have not been restored or subjected to overwhelming trauma. It is suspected that the DEJ structure plays a key role in preventing the transmission of cracks through the brittle enamel and into the tougher dentin. The apparent key role of the DEJ in forming this critical biomechanical complex between the dissimilar calcified tissues has prompted its study and comparative study of enamel and dentin.

1.2. Mechanical properties of enamel, dentin and the DEJ

Bulk properties of enamel and dentin have been studied by many investigators, with results that are quite variable (see Ref. 12 for review). This variability may stem in part from the variations in structure within dentin and enamel and the brittle and anisotropic nature of enamel. Recent work using AFM-based nanoindentation suggests that enamel has modulus and hardness values that vary from about 75–90 GPa, depending on orientation,[16] while the intertubular dentin normally has values of about 20 and 1 GPa for reduced elastic modulus and hardness, respectively.[6] The DEJ as noted above is a complex region of small size and irregular geometry that probably forms a graded interphase. This makes it especially difficult to study using conventional mechanical testing methods. Simple tensile, compression or shear tests are difficult because of the complex geometry that results in imprecise and non-uniform stress distributions. Pioch and Staehle[7] examined the shear strength of human and bovine teeth in the region of the DEJ and reported mean values of 39 and 37.4 MPa, respectively. It is of interest that they reported that all of the fractures occurred in dentin, never exactly at the DEJ, an indication of the difficulty associated with this problem. A more appropriate method of testing the mechanical characteristics of the DEJ requires a fracture mechanics approach. Rasmussen et al.[17] used a work-of-fracture (W_f) approach to determine the fracture characteristics of enamel and dentin. They found anisotropic W_f values for enamel, showing more resistance to fracture perpendicular (200 J/m^2) to the prisms than parallel to them (13 J/m^2). Dentin also was found to be anisotropic and more resistant to fracture, with values of 550 J/m^2 parallel to the tubules and 270 J/m^2 perpendicular to the tubules. The same technique was also applied to the region near the DEJ for which a value of about 336 J/m^2 was found in comparison to 221 and 391 J/m^2 for fracture perpendicular and parallel to the tubules, respectively.[8] It was noted that even with positioning the mandrel within 0.2 mm of the DEJ, minimal failure actually occurred at the DEJ, and it was concluded that the work of fracture of the dentin increases in the vicinity of the DEJ.

Lin[18] and Lin and Douglas[5] conducted extensive work on the fracture toughness values of bovine DEJ in an effort to establish if the DEJ offers a unique fracture resistant structure and therefore might indicate a basic principle of biomechanical design. They showed that the DEJ appears to have wider functional width (50–100 μm), than anatomic appearance, which may be related to mantle dentin; probably undergoes plastic deformation during crack propagation; and most likely serves as a crack deflector and blunter to prevent catastrophic tooth or enamel fractures. They reported substantially higher values of K_{IC} and G_{IC} for bovine DEJ than for bovine enamel or bovine dentin. In addition they found that substantial plastic deformation occurred as a crack crossed the DEJ. They concluded that enamel and dentin are optimized for different roles in response to complex stresses, and that they, along with the DEJ, act as an integrated biomechanical complex. Furthermore, they suggested that the DEJ can be regarded as a fibril-reinforced composite with moderate mineralization that is a complex zone capable of plastic deformation.[5] This is in contrast with the very brittle characteristics of the enamel and the tougher, but still somewhat brittle characteristics of dentin.[8] Cracks were propagated perpendicular to the DEJ in these studies, crack length was not measured, and the complexity of stresses as the crack propagated near and through the interface could not be determined.

2. Focus of recent work

The DEJ forms a complex biomechanical structure with a unique, but poorly defined multi-layered microstructure of at least three levels of uncertain organic and mineral content as compared to the adjacent tissues. There is no systematic information defining the variations with intratooth location or between teeth. In addition, mechanical properties have only been explored in limited fashion, because of the very small size and difficulty in isolating the DEJ for testing. However, the available fracture characteristics indicate that the DEJ differs substantially from either enamel or dentin, and probably provides a critical link that preserves the physical integrity of the tooth. Such a junction appears to confer critical properties allowing tooth structure to function efficiently. As such it may be optimized to its function of linking materials of substantially different properties. This suggests that the DEJ could serve as an important biomimetic model for linking other materials with dissimilar materials, a suggestion previously made by Lin and Douglas.[5] There are many applications for such an optimized junction, if it represents an optimized biomimetic design. These include dental and orthopedic implants, implant coating geometries, enamel and dentin bonding systems, and restorative dental interfaces in general. Our current work seeks to gain new insight into the structure and function of the DEJ with its potential biomimetic design principle in mind through the application of new methods that will permit a more comprehensive evaluation of its structural and mechanical properties at each of its levels of complexity.

2.1. DEJ scalloped microstructure

A high-resolution non-invasive method of imaging, Synchrotron Radiation Computed Tomography (SRCT), and chemical exposure of the DEJ by dissolution of the enamel, are two complimentary imaging approaches used to evaluate the DEJ structure.

3. SRCT

SRCT images were collected of tooth specimens bonded to composite restorative material, using synchrotron beamline 10-2 at Stanford Synchrotron Radiation Laboratory (SSRL), with a resolution of 3.33 μm. This method allows any selected X-ray attenuation level to be displayed and the attenuation level corresponding to the DEJ was selected and is shown in Fig. 5. This image of the DEJ clearly shows the scalloped structure in this non-destructive image.

4. DEJ scallops and sizes

A method has been developed to expose the DEJ by removing the enamel.[19] Human incisors and molars were sectioned buccal-lingually and the enamel was ground down to less than 1 mm and then removed with 0.5M EDTA (pH 7.4) until the DEJ was revealed, 7–10 days. The enamel was "chalky" in appearance when treated with EDTA, so the disappearance of this feature was an indication that the enamel was gone. Samples were fixed in gluteraldehyde and dehydrated in a graded ethanol series, followed by drying in HMDS, prior to sputter coating and examination in the SEM. Five SEM images were collected at 500 and 2000× from five areas in each the occlusal (incisal), middle and cervical thirds of each tooth. Diameters of scallops were measured in three lines (120° separation) for each scallop. Typical images for both tooth types are shown in Fig. 6. There were no significant differences with intratooth location, but the differences between tooth type were significant, with the average scallop size in incisors of 29.4 ± 5.5 μm and for 42.3 ± 8.5 μm molars (t-test, $P < 0.001$).[20]

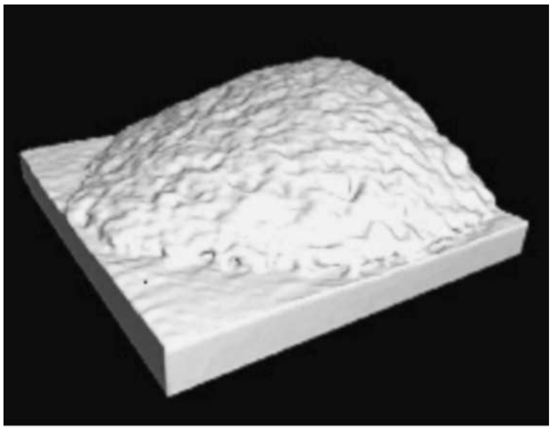

Fig. 5. SRCT image of DEJ in an uncut tooth, showing scalloped structure.

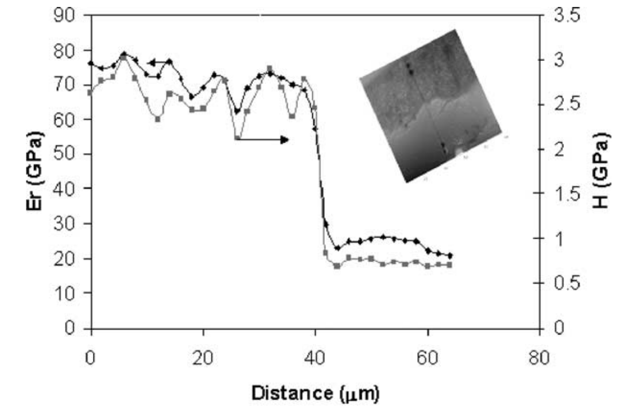

Fig. 7. AFM image showing AFM based indentations across the DEJ and associated plot of modulus and hardness.

4.1. Mechanical properties and functional width of the DEJ

Recent developments in AFM-based nanoindentation allow site-specific measurement of both reduced elastic modulus and hardness in nearly any environment. We used an approach in which the standard AFM head was replaced by a capacitive sensor allowing measurement of both load and displacement during indentation.[6] Indents can be placed along a line across the DEJ at 1–2 µm intervals, to avoid interactions between the indentations. Stiffness can be obtained from the unloading curve allowing calculations of reduced elastic modulus, E, while hardness can be determined from maximum force divided by the projected indent area, following the methods of Doerner and Nix.[21] Fig. 7 shows lines of indentations across the DEJ (inset) and typical results of the variation of reduced elastic modulus and hardness. These evaluations consistently show monotonically decreasing modulus values from about 70 GPa in enamel to 20 GPa in dentin.[6] Similarly, hardness decreases monotonically across the DEJ from approximately 3.5 GPa in enamel to about 1 GPa in dentin.

High-resolution images of the DEJ by a variety of imaging methods suggest there is a close apposition of the apatite crystals of dentin and the larger apatite crystals of enamel. This is shown in Fig. 8, a high resolution AFM image of the junction. In contrast with this observation, the alteration in mechanical properties occurs over a considerable distance. Thus the anatomic appearance and range of properties change may be quite different. The change in properties across the DEJ has been termed its functional width. Interestingly, studies using varying methods, e.g. fracture studies, and indentation studies, give quite different estimates of the functional width. Habelitz et al.[3] pointed out that the functional width appears to depend on indentation technique, indenter tip type, and load. Estimates from microindentation have given values of up to 100 µm,[22] while Berkovich and cube corner nanoindentation have suggested values of about 25 and 12 µm, respectively.[2,6]

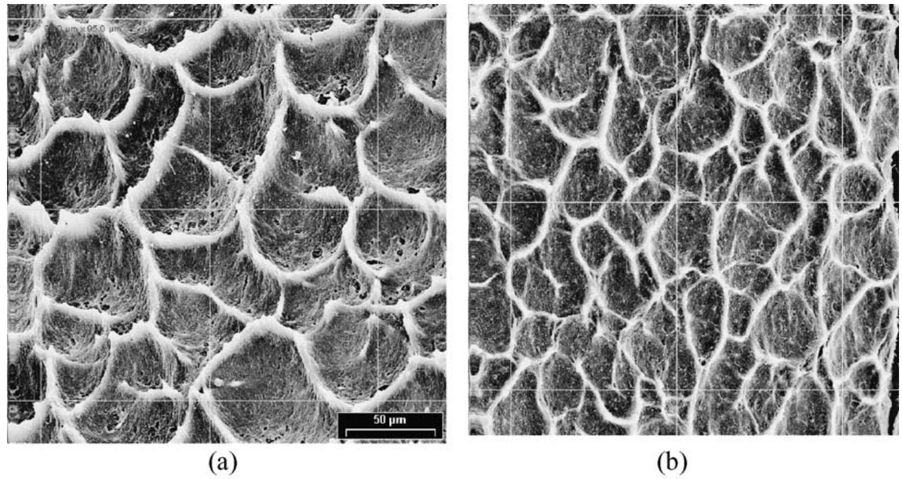

Fig. 6. SEM micrographs of the DEJ after removal of enamel, in a molar (a) and an incisor (b).

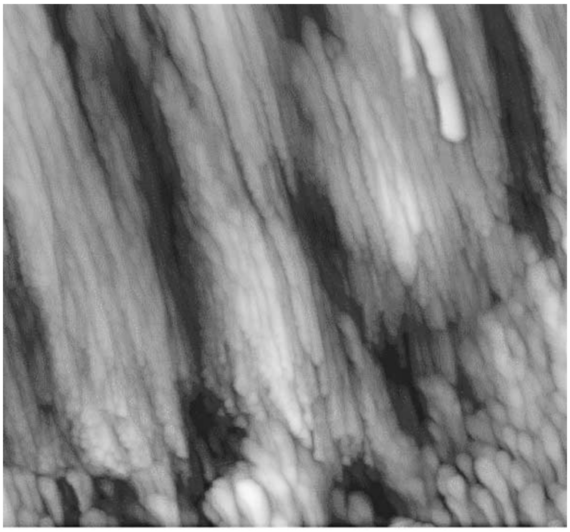

Fig. 8. High resolution AFM image of DEJ showing apatite crystals from the enamel (top) in close approximation to those in the dentin (bottom).

In an effort to resolve the nature of the functional width we recently employed a nanoscratching technique that provides continuous measurements of changes in friction or ploughing forces across the DEJ. A small spherical indenter attached to an AFM allowed the interface to be "scratched". Friction coefficients of enamel, dentin and at the DEJ were obtained with the nanoscratch tester attached to an AFM, that uses two perpendicular capacitive sensors to measure force and location in vertical and lateral directions. Normal loads in the range of 50–600 μN were applied to a spherical diamond indenter ($r = 10$ μm), which was driven across the sample surface, recording the lateral force. Imaging by AFM facilitated exact positioning of the scratches (see inset shown in Fig. 9). Fig. 9 shows that values of friction coefficients in enamel and dentin were about 0.31 and 0.16, respectively. Further measurements on a variety of teeth at varying loads gave a friction coefficient of intertubular dentin as 0.31 ± 0.05, significantly above the coefficient of enamel (0.14 ± 0.02).[3] The difference in friction coefficients may have been a result of the higher protein content of dentin. Scratches across the DEJ showed a sharp monotonic change in the friction coefficient over a smaller distance than discrete indentation methods, as seen in Fig. 9. The average width of the slope between the friction coefficients of dentin and enamel was 2.0 ± 1.1 μm, that may be an improved estimate of the functional width of the DEJ. Because the tip used for nanoscratching had a radius of 10 μm the true value of DEJ functional width may be even less than the 2 μm determined with this technique.

A variety of other methods is available to estimate the change in properties across the DEJ and obtain additional estimates of the functional width. For example, Gallagher et al.,[23] used 351-nm laser excitation to study the DEJ by autofluorescence microscopy and found the median DEJ width was approximately 10 μm, in good agreement with nanoindentation results. Another approach that might prove useful for determining functional width is measurement of compositional variation across the DEJ. We recently employed microRaman spectroscopic imaging to evaluate the DEJ and estimate its width based on changes in mineral and organic content across the junction.[24] Peak positions

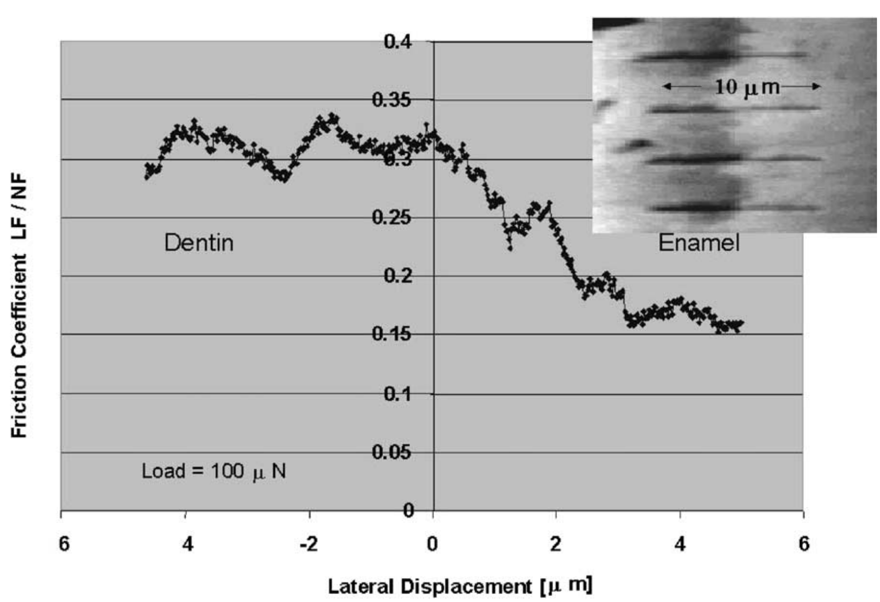

Fig. 9. Plot of friction coefficient across DEJ, determined from nanoscratching in the AFM, with scratches shown in the inset. Average friction coefficients were 0.31 in dentin and 0.16 in enamel.

and intensities were determined from micro Raman spectra for PO_4^{3-} band and the C–H stretching modes and were compared among the mineralized tissues and their junctions. Samples were subjected to monochromatic radiation (632.8 nm) emitted by He–Ne laser focused to about 0.5 μm spot size, and spectra were measured in 1 μm steps along 100 μm lines across the DEJ. The intensity variations for the PO_4^{3-} and C–H stretching modes across the DEJ are shown in Figs. 10 and 11. The mineral content monotonically decreased from enamel to dentin, while the organic component monotonically increased. The functional width was estimated from the intersections of regression lines fit to the intensity values (enamel, DEJ, dentin) vs. distance across the junction. The PO_4^{3-} band gave a value of 7.6 (±2.8) μm, while the C–H stretching mode yielded 8.6 (±3.6) μm. High-resolution analysis indicated no difference in peak position for the PO_4^{3-} band among the tissues (959 cm^{-1}). Enamel showed a 4.6 cm^{-1} peak shift in the C–H stretching mode as compared to DEJ or dentin, but no unique components could be found in the DEJ. This suggests that there is a continuous gradient of organic components across the DEJ.

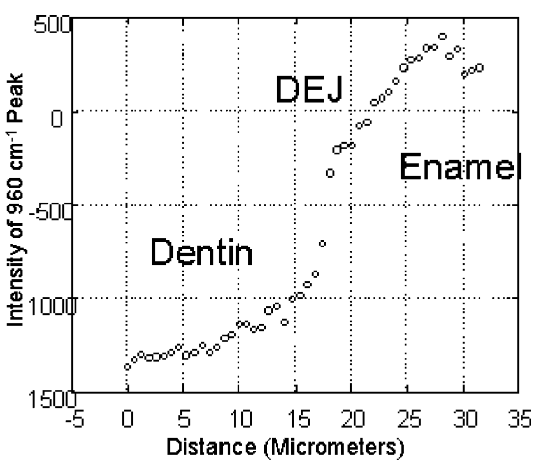

Fig. 10. Plot of Raman intensity for peak associated with PO^{4-} at 960 cm^{-1}. High intensity levels were present in enamel on the right, with low levels in dentin, on the left. The estimated DEJ width was 5–7 μm from this method.

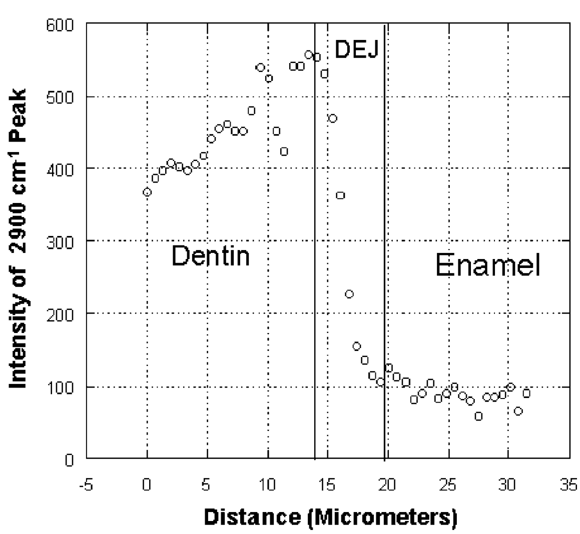

Fig. 11. Plot of Raman intensity for peak associated with C–H stretch at 2900 cm^{-1}. High intensity levels were present in dentin on the left, with low levels in enamel, on the right. The estimated DEJ width was 5–7 μm from this method.

It may be useful to ask the meaning of functional width and factors that might influence the estimates using different techniques. There is little evidence to suggest that this junction has a unique composition that causes the observed variations in properties. Because of the three-level scalloped microstructure, variations in properties from nearly any method that probes the DEJ in cross-section can be explained based on the relative proportions of enamel and dentin that are probed at each point. This model suggests that different estimates of the functional width result from the scalloped morphology of a very narrow junction between enamel and dentin. This conclusion is further reinforced by the monotonic variation of organic and mineral content across the junction, and by the fact that no unique peak shifts are seen in this region. Thus it appears that inter-digitation of enamel and dentin expressed at three length scales: scallops, micro-scallops, and nano-level features can account for many of the properties exhibited by the DEJ.

5. Fracture properties of the DEJ

Perhaps the fracture and crack deflecting characteristics of the DEJ are of most interest as they may reveal unique mechanisms that may be useful in efforts to join dissimilar materials. So far these characteristics have proved elusive. Rasmussen[17] first noted that it was extremely difficult to propagate fractures at the DEJ, and similar results have been reported more recently by Pioch and Staehle[7] in shear strength studies, as well as in fractures induced by microindentation by Xu et al.[25] and nanoindentation by Marshall et al.[6] Fig. 12 shows a crack initiated in enamel near the DEJ using nanoindentation that failed to cross the DEJ, but rather was deflected and propagated just inside the enamel. Cracks could not be initiated up to a maximum load of 30,000 μN on the dentin side of the junction, with a cube corner diamond tip. Thus the fracture characteristics of the DEJ and the local influence of the DEJ architecture on these characteristics need additional study, as they are most likely to give important insights into its most intriguing functional characteristic.

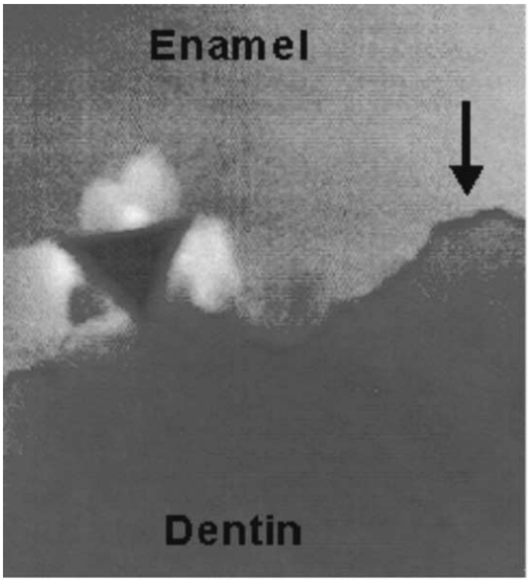

Fig. 12. AFM image showing indent in enamel that caused a crack that approached the DEJ, but deflected and propagated in the enamel.

Acknowledgements

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