Three-dimensional Changes in Direction and Interrelationships among Enamel Prisms in the Dog Tooth

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Summary. This study describes the three-dimensional features of enamel prisms and their arrangement in dog teeth. Tangential semithin sections of demineralized tooth germ were serially cut from the enamel surface to the enamel-dentin junction. Straight rows of enamel prisms parallel or perpendicular to the meridian were selected at the enamel-dentin junction; these prisms were reconstructed from micrographs with a personal computer.

Near the enamel-dentin junction, the arrangement of enamel prisms appeared regular. Viewed from the enamel surface, the cut-ends of the enamel prisms that were parallel to the meridian at the enamel-dentin junction appeared as a sine curve, with 16 enamel prisms forming one period. The enamel prisms in a row perpendicular to the meridian were parallel to each other and deflected to the left or right from the enamel-dentin junction. Away from the enamel-dentin junction, the periodicity of the prisms gradually disappeared. The sine curve formed by the cut-ends of prisms in a row parallel to the meridian became irregular, and prisms in rows perpendicular to the meridian crossed each other.

The semithin sections showed belt-like zones arranged perpendicular to the meridian. Each belt-like zone consisted of enamel prisms oriented in the same direction, those in neighboring zones being oriented in opposite directions. The disappearance of the regular arrangement of prisms was related to changes in their location in the belt-like zones.

It is generally accepted that enamel prisms stretch nearly perpendicularly from the enamel-dentin junction, curving horizontally and tilting longitudinally. These three-dimensional features of enamel prisms have been examined by many workers using a variety of reconstruction techniques (Yoshida, 1938; Osborn, 1967, 1968a, b; ABE et al., 1991). From the serial photographs of this through-focussing technique, enamel prisms were reconstructed with a wax model (Osborn, 1967) and three-dimensional computer graphics (ABE et al., 1991). Though the through-focussing technique is very useful, it does not allow observation over long distances of enamel prisms extending from the enamel-dentin junction.

Three-dimensional reconstructions of prisms have been made from serial sections of decalcified enamel (Warshawsky and Smith, 1971; Hanaizumi et al., 1992). Warshawsky and Smith (1971) reconstructed two enamel prisms from serial cross sections of rat incisor enamel with a resin model. Showing the interrelationship between prisms has remained impossible, however, because of difficulties in reconstructing enamel prisms from oblique sections of prisms.

Recently, Hanaizumi et al. (1992) have reconstructed enamel prisms by computer simulation after cutting serial tangential sections of demineralized dog tooth germ from the enamel surface to the enamel-dentin junction. Their graphic representation showed the three-dimensional course and the interrelationships between enamel prisms extending from the enamel-dentin junction. However, their reconstructions of prisms were limited to the area near the enamel-dentin junction. The present study aims to investigate the three-dimensional changes in direction and the interrelationships among prisms by the reconstruction of prisms extending from the enamel-
dentin junction to the surface.

MATERIALS AND METHODS

Light microscopy
A one-month-old dog was used for the study. It was perfused with 0.1 M phosphate buffered solution of 2% paraformaldehyde and 1.25% glutaraldehyde at pH 7.4. The jaws were removed and immersed in the same fixative overnight. Permanent tooth germs were dissected out and demineralized in 5% EDTA (pH 7.4) for three weeks.

For light microscopy, tooth germs were postfixed in 1% osmium tetroxide for 3 h, dehydrated in ethanol, and embedded in Spurr's resin. First the tooth germ was cut with glass knives in a direction parallel to the tooth axis until the longitudinal plane vertical to the enamel-dentin junction was obtained. The block was remounted on the ultramicrotome at right angles to the original sectioning plane with the tooth axis unchanged. Serial sections were produced parallel to the tooth surface in the direction toward the enamel-dentine junction (Fig. 5a). They were stained with methylene blue and azure II, and photographed with a light microscope (Nikon Microphot-FXA Tokyo).

Three-dimensional reconstruction
As reference points for reconstruction, two holes in the resin were made by a pin fixed at right angles to the sectional plane. The contours of selected prisms were reconstructed by tracing these two holes on enlarged photoprints of sections. The data were stored in a personal computer (PC-9801 VM NEC) equipped with three-dimensional graphic software (Cosmozone 2SA, Nikon) for the reconstruction of enamel prisms.

RESULTS

Light microscopy
In a longitudinal section vertical to the enamel-dentin junction, Parazones and Diazones of Hunter-Schureger bands were shown in alternate rows at 50 μm intervals (Fig. 1). Serial tangential sections produced from the tooth surface were used for reconstruction.

Since the planes of sections were considered to be parallel to the enamel-dentin junction near the exposed dentin areas, enamel prisms for reconstruction were selected from such areas (Fig. 2). Enamel prism sheaths were difficult to determine at the enamel-dentin junction, and a section 3 μm apart from the enamel-dentin junction was used as the initial plane for reconstruction of the enamel prisms (Fig. 3). A straight row of enamel prisms parallel to the meridian was selected for reconstruction, and each prism was numbered from the top (Fig. 3a). Similarly, two straight rows of prisms perpendicular to the meridian crossing the parallel row at Prism 11 and 21 as shown in Figure 3a were also selected for reconstruction, and numbered from the right-hand side (Fig. 3b).

At 50 μm apart from the enamel-dentin junction, the enamel prisms initially parallel to the meridian formed a wavy sine curve (Fig. 4a). The enamel prisms initially perpendicular to the meridian remained in a nearly straight line (Fig. 4b). The enamel prisms used for reconstruction were limited to odd-numbered ones because of memory limitation of the computer (Figs. 3, 4).

Reconstruction of enamel prisms

Prisms parallel to the meridian
Reconstruction at 70 μm from the enamel-dentin junction: A row of enamel prisms parallel to the meridian was reconstructed 70 μm from the enamel-dentin junction (Fig. 5b). Viewed from the enamel surface, the cut-ends of the enamel prisms formed a wavy sine curve with 16 enamel prisms constituting one period. The Prism 3 (red), 11 (pink), 19 (light blue), and 27 (yellow) were at the extreme point of the wavy curve, tilting most extensively to the enamel-dentin junction (Fig. 6a).

Reconstruction at 170 μm from the enamel-dentin junction: Viewed from the surface, the wavy curve formed by the cut-ends of these enamel prisms 170 μm from the enamel-dentin junction showed no periodic pattern. The cut-ends of the colored prisms were not at the extreme points of the wavy curve approaching the surface (Fig. 6b).

Lateral aspects of the prisms were perpendicular to the enamel-dentin junction and nearly parallel to each other near the enamel-dentin junction. Some started to tilt to the top when approaching the surface (Fig. 6c).

When viewed from the cervical direction, the red and light blue prism and the pink and yellow prism overlapped near the enamel-dentin junction. Approaching the surface, these colored prisms moved from the extreme point of the wavy curve (Fig. 6d).

Prisms perpendicular to the meridian
Two straight rows of enamel prisms perpendicular to the meridian at the level of the enamel-dentin junction were reconstructed 150 μm apart from the
enamel-dentin junction, and viewed from a cervical direction. The upper row (as shown in Figure 3b) ran parallel, tilting to the left immediately after emerging from the enamel-dentin junction. Approaching the surface, some of the prisms changed the angle of tilt and extended themselves perpendicular to the enamel-dentin junction. Some crossed the paths of other prisms, and others bent to the right (Fig. 7a).

The lower row (as shown in Figure 3b) ran parallel, perpendicular to the enamel-dentin junction. Approaching the surface, the right side prisms tended to tilt to the right (Fig. 7b).

Fig. 1. Micrograph of a longitudinal semithin section of a canine tooth cut parallel to the tooth axis. Parazone (p) and diazone (d) of Hunter-Schreger bands alternating at intervals of 50 μm. E enamel, D dentin, Ab ameloblast. x135

Fig. 2. Micrograph of a tangential section at the level of the enamel-dentin junction. The dentin area (D) is considered to be at a right angle to the enamel-dentin junction, and prisms for reconstruction were selected from this area. The holes on the right (arrowheads) were made in the resin block with a pin fixed at right angles to the sectioning plane; prisms were reconstructed by using these two holes as reference points. E enamel, D dentin, Ab ameloblast. x107
Belt-like zone

Light microscopy

The semithin section cut at 50 \( \mu \text{m} \) from the enamel-dentin junction showed many belt-like zones arranged roughly perpendicular to the meridian (Fig. 8a). The direction of enamel prisms here could be estimated from the inclination of the top of the prisms sheaths, and indicated that each belt-like zone consisted of enamel prisms oriented in the same direction (Fig. 8b), with neighboring zones being oriented opposite. The boundaries between two neighboring zones consisted of groups of round, small prisms (Fig. 8b).

Reconstruction of the belt-like zone

The boundaries of the belt-like zones were reconstructed at planes 50–150 \( \mu \text{m} \) apart from the enamel-dentin junction, and viewed from the surface (Fig. 9a). The boundaries of the belt-like zones were of equal width and parallel, especially near the enamel-dentin junction. Away from the enamel-dentin junction, the boundaries of the belt-like zones became irregular. The reconstructed images of enamel prisms in Figures 6b, 7a, b were superimposed on the belt-like zones in Figure 9a (Fig. 9b–d). The reconstructions of the boundaries of the belt-like zones and the row of prisms parallel to the meridian revealed the three-dimensional interrelationship between the belt-like zones and the prisms. In a single belt-like zone,
the horizontal tilt angles of the enamel prisms towards the enamel-dentin junction showed a tendency to be greatest at the center, and least near the boundaries (Fig. 9b). The rows of prisms, perpendicular to the meridian, located at the center of the belt-like zones were parallel near the enamel-dentin junction (Fig. 9c, d). The prisms tended to run perpendicular to the enamel-dentin junction when approaching the boundaries of the belt-like zones. One prism twisted to run in the opposite direction after crossing the boundary of the belt-like zone (Figs. 7a, 9c). Similarly, the prisms showed a tendency to tilt towards the enamel-dentin junction when approaching the center of the belt-like zone (Figs. 7a, 9d).

**DISCUSSION**

The periodic pattern in arrangements of enamel prisms

Reconstructions of enamel prisms have been produced by various methods (Yoshida, 1938; Osborn, 1967, 1968a, b, 1971; Warshawsky and Smith, 1971; Abe et al., 1991; Hanaizumi et al., 1992); some investigators have reported periodic patterns in the arrangement of the enamel prisms (Osborn, 1968a, b, 1970, 1973; Hanaizumi et al., 1992). Osborn (1968a, b, 1970, 1973) studied relatively thick untreated ground sections of human enamel by a through-focusing technique to determine the directions and interrelation-
ships of prisms, and suggested changes in direction of about 2° between successive horizontal rows of prisms, transverse to the long axis of the tooth. In his study, prisms were maximally inclined 10° from a radial direction, and changed direction in cycles of 20 prisms. As a result, the prisms in the vertical rows appeared to wave around their long axis parallel to the tooth, and a half cycle of prisms constituted a band of Schreger.

Hanaizumi et al. (1992) reconstructed enamel prisms of dog teeth with computer graphics to examine the three-dimensional course and the interrelationship between prisms up to 75 μm from the enamel-dentin junction. Similar to Osborn (1968a, b, 1970, 1973), this demonstrated that rows of prisms originally straight and parallel to the meridian of the tooth showed a periodic tilting pattern. The tilt angle between neighboring groups of enamel prisms was 8°, alternating at eight prism intervals. From the enamel surface, the cut-ends of enamel prisms appeared as a wavy sine curve, with 16 enamel prisms forming one period. However, the three-dimensional course and the interrelationship between prisms showed a tendency to become irregular as the distance from the enamel-

Fig. 5 a. Diagram showing the position of the serial semithin sections and their directions in the three-dimensional reconstruction. b. Three-dimensional reconstructions of the odd-numbered enamel prisms in Figure 3a extending 70 μm from the enamel-dentin junction. Viewed from the same direction as in Figure 5a.

Fig. 6. Reconstruction of the odd-numbered prisms in Figure 3a extending 70 μm (a) and 170 μm (b-d) from the enamel-dentin junction, respectively. a. Prisms are reconstructed up to 70 μm from the enamel-dentin junction. Viewed from the enamel surface. The tilt of the enamel prisms alternates at eight prism intervals. The eight prisms of a group show a periodic tilting pattern. The ends of the enamel prisms appear as a wavy sine curve, with 16 enamel prisms forming one period. The red, pink, light blue, and yellow prisms correspond to Prisms 3, 11, 19 and 27 in Figure 3a, respectively, and are tilted most horizontally. b. Prisms reconstructed up to 170 μm from the enamel-dentin junction. Viewed from the enamel surface. The periodic wavy curve pattern of the cut-ends of the enamel prisms seems to have disappeared. The cut-ends of the colored prisms are not at the extremes of the curve. c. Prisms reconstructed up to 170 μm from the enamel-dentin junction. Lateral view. The prisms run perpendicular to the enamel-dentin junction and are nearly parallel near the enamel-dentin junction. Approaching the surface, some of prisms tilt to the top of tooth (arrowheads). d. Prisms reconstructed up to 170 μm from the enamel-dentin junction. Vertical view. Near the enamel-dentin junction, the red and light blue prism, and the pink and yellow prism overlap (bars). Near the surface, the position of the colored prisms shifts from the extremes of the wavy curve.
Fig. 7. Reconstructed rows of enamel prisms perpendicular to the meridian extending 150 μm from the enamel-dentin junction. Vertical view. **a.** The upper row of parallel prisms in Figure 3b, tilted left immediately from the enamel-dentin junction (arrowheads →). Near the surface, some prisms change angle of tilt and extend perpendicular to the enamel-dentin junction, crossing the paths of other prisms (arrowheads ↑), while some bend in the right direction (arrow). **b.** The lower row of parallel prisms in Figure 3b, perpendicular to the enamel-dentin junction (arrowheads →). Near the surface, the right side prisms tilt right toward the enamel-dentin junction (arrowheads →).
Fig. 8. Tangential semithin section 50 μm from the enamel-dentin junction. a. The semithin section shows many belt-like zones roughly perpendicular to the meridian. ×125. b. Enlargement of Figure 8a. The belt-like zones each consist of groups of prisms oriented in the same direction. Right pointing arrowheads (►) show groups of prisms oriented to the right. Left pointing arrowheads (◄) show groups of prisms oriented to the left. There are groups of round, smaller prisms between neighboring zones (★). ×315
Fig. 9. Legend on the opposite page.
dentin junction increased (Hanaizumi et al., 1992).

This study attempted to reconstruct the full length of prisms from the enamel-dentin junction to the surface. It was found that the regular arrangement between the rows of enamel prisms parallel or perpendicular to the meridian of the dog tooth disappeared over 70 µm away from the enamel-dentin junction. This may be explained as follows: Near the enamel-dentin junction, the enamel prisms display only a sidewards displacement in addition to radial movement. Approaching the surface, some of the enamel prisms display vertical displacement in addition to the sidewards displacement. As these prisms are displaced vertically to a certain degree in the tooth enamel, the prisms alter the amount of sidewards displacement. For this reason, the wavy curve formed by the cut-ends of prisms parallel to the meridian has no periodic patterns.

Interaction between the boundaries of the belt-like zones and sidewards displacement of enamel prisms

Using tangential ground sections and reflected light microscopy, previous investigators have shown belt-like zones, with each consisting of groups of enamel prisms oriented in the same direction—in dog (Süss, 1940) and human (Wolf, 1942) enamel. Süss (1940) provided a schematic model showing the distribution of belt-like zones and three-dimensional course of the enamel prisms in the belt-like zone. Wolf (1942) further illustrated the movement of ameloblasts in the belt-like zones displaying the alternating sidewards movement of ameloblasts to from a wavy curve. These schematic models were insufficient to represent the three-dimensional arrangement of the enamel prisms, because they were based on observations from only two directions. The present study attempted to reconstruct the boundaries of the belt-like zones, and to establish the spatial relationship between the belt-like zones and the sidewards displacement of the enamel prisms.

The present study showed that the enamel prisms of each belt-like zone displayed similar sidewards displacement, and that the direction of displacement in neighboring zones was opposite. This corroborates reports of the three-dimensional model of enamel and its relation to the bands of Schreger (Gustafson, 1945; Erausquin, 1949; Kawai, 1951, 1955; Applebaum, 1960; Heuser, 1961; Skobe, 1980; Hirota, 1982). The present study reaffirmed that the sidewards displacement of enamel prism is greatest at the center of the belt-like zones and becomes smaller near the boundaries of the belt-like zone (Fig. 9b).

The present study also showed that the arrangement of enamel prisms become increasingly irregular when vertical displacement was combined with sidewards displacements. With vertical displacement, the position of a certain enamel prism in relation to the belt-like zone is changed and, hence, the prism comes to run at a different horizontal angle of tilt, causing changes in the sidewards displacement. This would explain why the red, pink, light blue, and yellow prisms in Figure 6 alter their horizontal tilt to the enamel-dentin junction as they approached the surface (Fig. 6b, d). These colored prisms displayed the greatest sidewards displacement and horizontal tilt near the enamel-dentin junction, because the individual prisms were at the center of the respective belt-like zones. Nearer the surface, these prisms approach the boundaries of the belt-like zone with reduced sidewards displacement (Fig. 9b). Consequently, these prisms run at right angles to the enamel-dentin junction with decreasing horizontal angles of tilt (Figs. 6b, 9b). Moreover, the prism displays the opposite sidewards displacement after crossing the boundary between the belt-like zones (Figs. 7a, 9c). Similarly, the sidewards displacement of the prism increases near the center of the belt-like zone, resulting in a larger horizontal tilt to the enamel-dentin junction (Figs. 7b, 9d).

To elucidate the mechanisms of the formation of the Schreger bands, a precise knowledge of the spatial movement of ameloblasts as well as the three-dimensional course of the enamel prisms will be needed.

Fig. 9. Model of the three-dimensional structure of the belt-like zones and enamel prisms, viewed from surface. a. Reconstructed boundaries of belt-like zones extending 50–150 µm from the enamel-dentin junction. Arrowheads show the orientation of the prisms in each belt-like zone. b. A combination of belt-like zones with the enamel prisms shown in Figure 6b. The horizontal tilt angles of the enamel prisms tend to be greatest at the center of a belt-like zone and smaller near the boundaries. The prisms tend to run at right angles toward the enamel-dentin junction near the boundaries of the belt-like zone (arrows). c. A combination of belt-like zones with the enamel prisms shown in Figure 7a. The prisms tend to change direction when they cross the boundaries of the belt-like zones (arrow). d. A combination of belt-like zones with the enamel prisms shown in Figure 7b. Rightward tilting toward the enamel-dentin junction increases as the enamel prism approaches the center of the belt-like zone (arrowhead).
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