Microvascular Architecture of the Enamel Organ of the Upper Major Incisor in the Rabbit

By

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Summary: The ultrastructure of the ameloblasts in the rabbit major incisor was investigated previously by Okada (1983) and the amelogenetic process was classified into six zones/stages. The present paper deals with changes in the microvascular architecture and ultrastructure of the blood capillaries in proportion to the amelogenetic process in the upper major incisor of the rabbit utilizing the acryl plastic injection method.

Three different vascular layers were observed in the periodontal spaces of the major incisor of the rabbit. The inner vascular network consisted of a capillary network supplying the enamel organ and its meshes have vigorously changed during the amelogenesis. The capillary network was observed to be in the shape of a ladder with a continuous wall in the proliferation zone, to appear as round meshes with a fenestrated wall in the differentiation zone, as polygonal meshes with abundant fenestrations in the secretion zone, as ovoid meshes with fenestrations in the early maturation zone, and finally as coarse and ovoid meshes with a continuous wall again in the late maturation and regression zones. In the intermediate layer, arterioles and venules were located close to the capillary network, and the arterioles were derived from the short and long branches of the anterior superior alveolar artery. In the outer layer, a sinusoid network was observed to be in contact with the alveolar wall and received blood from the capillary network as well as venous vessels in the alveolar bone.

The ladder-shaped capillary network mentioned above was thought to represent an intermediate form towards the succeeding zone, in which the round meshes may be suitable for supplying the nutrient elements that are needed in the differentiation of the inner enamel epithelial cells. The polygonal and ovoid meshes may be favorable for the transport of various necessary metabolic materials that are involved in the enamel ground substance formation and calcium deposition within a very short period.

It is important and significant to elucidate the relations between the ameloblasts and the microvascular changes in various zones/stages of the amelogenesis in the permanently growing tooth, because all periods (i.e. during the developing, maturating and regressive processes) of the amelogenesis are successively supplied by metabolic materials from the blood stream. Nevertheless, a majority of archives concerning the development of the tooth have concentrated on the cytological elements of the tooth germ, although a gross angiological approach to the tooth germ and its vicinity was adopted by Tasumi (1965a and b) and by Iwaku et al. (1979).

The present authors attempted to elucidate the serial changes of the microvascular architecture three-dimensionally during six zones/stages presented by Okada (1983), as shown in Fig. 1, during the amelogenesis of the rabbit major incisor, utilizing the acryl plastic injection method to investigate the microvascular architecture. Simultaneously, ultrastructural observations were made of changes to the vascular wall in each zone.

Material and Methods

Fifteen adult rabbits were used in this study. Ten of the rabbits were injected with acryl plastic via the common carotid arteries by the plastic injection method of Ohta et al. (1990). The incisors including surrounding tissues were digested with 10% sodium hydroxide to prepare microvascular casts. These were coated with gold employing an ion sputter coater (JFC-1500, JEOL, TOKYO) and observed under a scanning electron microscope (JSM-T300, JEOL, TOKYO).

The remaining five rabbits were perfused from the common carotid arteries with 2.5% glutaraldehyde in 0.1 M phosphate buffer for 2 hours. The upper incisors
were dissected out, decalcified in 5% EDTA at 4°C for one week, and postfixed in 1% osmic acid. The materials were ultrathin-sectioned after embedding in SPURR resin, stained with uranyl acetate and lead citrate, and observed under a transmission electron microscope (JEM-100S, JEOL, TOKYO).

**Findings**

Three kinds of vascular layers were noted in the periodontal space on the labial side of the upper major incisor of the rabbit. The inner layer was composed of a capillary network located close to the enamel organ. The intermediate layer was composed of arterioles of the short and long branches (Deguchi 1977) of the anterior superior alveolar artery, and venules which drained from the capillary network running in parallel with the tooth axis. The outer layer was composed of a sinusoidal network located close to the labial alveolar wall (Figs. 2, 3, 4 and 5). This network drained into veins in the osseous tissue via foramina (Figs. 2, 3, 6a and 6b).

1. **Proliferation zone/stage (Fig. 1)**

The arterioles in this zone were located at the site of the enamel organ and spread into capillaries (Figs. 2 and 3). The capillary network extended in a flat plane with meshes which consisted mainly of capillaries with thick walls running in the tooth axis, and which communicated with a few cross-running twigs in the shape of a ladder. The blood in the capillary network drained directly into a sinusoid (venule) network locating in the developing end of the enamel organ (Figs. 7a and 7b). The nucleus of the endothelial cells of the above blood capillary protuded slightly and the cytoplasm was generally thin. The endothelial wall appeared to be continuous-typed with a basement membrane and pericytes. The basement membrane was arranged on both the capillary and outer enamel epithelial sides, so that it was observed as a double-layered structure in contact with the outer enamel epithelium (Fig. 7c).

2. **Differentiation zone/stage (Fig. 1)**

The arterioles in this zone usually took a course rather closer to the alveolar wall (Figs. 2 and 3) as compared to those in the previous zone. The outer enamel epithelium had begun to assume an undulated arrangement, holding blood capillaries within it (Fig. 8a). The capillary lumina appeared as a chain of swellings with smaller and round meshes. The capillaries had started to become meandering during the late stage (Fig. 8b). The blood from the capillary network drained into the sinusoid network in the outer layer via venules in the intermediate layer (Fig. 2). Although the cytological aspect of the endothelial cell were almost the same in.
this zone as those in the previous zone, the fenestrations observed in the late differentiation zone had started to become visible so as to alter the fenestrated capillaries. Microvilli-like projections and pinocytotic vesicles were observed (Fig. 8c). The basement membrane of the endothelium also appeared to be double-layered participating in that of the outer enamel epithelium (Fig. 8c).

3. Secretion zone/stage (Fig. 1)

The arterioles in the previous zone passed beyond the alveolar wall into the present zone and ran straight without branching. They spread radially into capillaries on the enamel organ (Figs. 2 and 3). These capillaries extended in a meandering fashion between tall ridges composed of the outer enamel epithelium to form a polygonal-meshed network by anastomosing with one another (Figs. 9a and 9b). These meshes had started to become elongated to ovoid meshes along the tooth axis in the area between the margin of the enamel organ and the labial central groove in the late secretion zone. The blood from the capillary network drained into venules in the intermediate layer (Figs. 2, 6a and 6b). These venules of various calibers meandered towards the developmental end anastomosing repeatedly with adjacent ones, and drained into the sinusoid network in the outer layer. The cytoplasm of the endothelial cells was extremely thin, with fenestrations. Abundant pinocytotic vesicles were observed in the non-fenestrated areas. The double-layered basement membrane found in the previous zone was also clearly observed (Fig. 9c).

4. Early maturation zone/stage (Fig. 1)

The arterioles passed straight forwards close to the alveolar wall in the middle of the enamel organ without ramifications up to its surface, where they spread into capillaries in a radial fashion and passed between papillae which consisted of papillary cells (Fig. 2). The meshes of the capillary network appeared to be elongated ovoid in the tooth axis. The blood from the capillary network, similarly to that in the previous zone, drained into the sinusoid network in the outer layer via venules in the intermediate layer (Figs. 10a and 10b). The endothelial cells and pericytes were similar to those of the previous zone in their morphological aspects, with a double-layered basement membrane (Fig. 10c).

5. Late maturation zone/stage (Fig. 1)

This zone occupied the widest area between the other five zones and was supplied by the ascending branch of the medial alveolar branch (Deguchi 1977) (Fig. 2). After arterioles passed in the center of the enamel organ without branching-off, they repeated to divide in a Y-shaped fashion before spreading into capillaries. The ascending branch passed upwards on the medial side of the periodontium up to the enamel organ and gave off arterioles in the tooth axis (Fig. 2). The capillaries passed straight, close to the papillary cells, in a flattened manner with small calibers (5 μm) and formed a network with elongated and coarse meshes in the tooth axis (Figs. 11a and 11b). The blood from the capillary network drained into venules in the intermediate layer and immediately into the sinusoid network in the outer layer. The cytoplasm was still thin including a few pinocytotic vesicles without fenestrations. The capillary wall changed to be continuous-typed, although the double-layered basement membrane was still visible (Fig. 11c).

6. Regression zone/stage (Fig. 1)

Arterioles which diverged from the ascending, long and short branches as found in the previous zone were observed in the early regression zone, but were later supplemented by those which diverged from arterioles supplying the inner marginal epithelium (Fig. 2). The meshes of the capillary network were noticeably coarse and elongated in the tooth axis (Figs. 11a and 11b). The meshes gradually changed to become smaller, rather denser and round at the attached epithelium. The blood from the capillary network drained into the venous network in the outer layer via venules. The capillary wall appeared to be continuous-typed with a double-layered basement membrane only at the area close to the papillary cells (Fig. 11c).

Discussion

There have been few works investigating the relationship between the vascular changes occurring with tooth development and the morphological changes of the ameloblasts. However, Tasumi (1965a and b) examined the ageing changes of the vasculature supplying the upper and lower incisors in the rat utilizing the india ink injection method under a light microscope. Kallenbach (1966 and 1967) observed that the capillary network of the papillary cell layer contributed to the process of enamel maturation. Iwaku et al. (1979) investigated the vasculature of the enamel organ of the lower incisor in the rat by means of plastic injection and attempted to elucidate the relations between various vascular changes in proportion to the amelogenesis in four zones. Yoshida et al. (1984 and 1985) examined the blood supply of the tooth germ of the rat molars three-dimensionally. Deguchi (1977) studied the blood supply of the upper major incisor, especially the ramifications of the anterior superior alveolar artery and their distribution features under a binocular microscope.

The present authors attempted to elucidate the relations between the microvascular changes occurring during the entire amelogenetic period and the morpho-
logical changes of the ameloblasts, according to the classification reported previously by Okada (1983).

1. Proliferation zone/stage

The nutrient vessels for the enamel organ in this zone were supplied from the dental papillary cell site, as suggested by Reith (1967) and Okada (1983). This may be why the mitochondria of the inner enamel epithelial cells were usually concentrated close to the distal or the dental papillary cell site. The present authors found that the capillary network with coarse meshes in the shape of a ladder, arranging in parallel to the tooth axis, consisted of continuous walls, with no pinocytotic vesicles contributing to the transport of metabolic materials, and separating the inner enamel epithelial cells from the capillary network by the existence of a wide stellate reticulum. Accordingly, the capillary bed surrounding the enamel organ existed only as a route to the next differentiation zone, and the supply route for the inner enamel epithelium may emanate from the dental papillary cell site.

2. Differentiation zone/stage

The mitochondria of the inner enamel epithelial cells were concentrated in their basal site. Such a concentration indicates that the nutrient supply from the dental papillary cell site may be excluded due to the commencement of predentin formation and transferred to the capillary site surrounding the enamel organ. This may be substantiated by the extension of the capillary network into the interridges of the outer enamel epithelium and a sudden decrease in mass of the stellate reticulum. Moreover, the capillary bed itself comes close to the inner enamel epithelium. In the late differentiation zone, these epithelial cells suddenly differentiate into ameloblasts and the capillary wall begins to change to become fenestrated. Such serial changes may presumably be involved in the transport of metabolic materials for the differentiation mentioned above.

3. Secretion zone/stage

In proportion to the formation of the enamel matrix in this zone, ameloblasts appear as typical, protein-secreting glandular cells which require a rapid nutrient supply. Accordingly, the meandering of the capillaries becomes stronger with fenestrations and the meshes become denser. Since the cellular processes of the outer enamel epithelium which are arranged in a table-shaped fashion to produce diaphragmatic structures, being close to the capillary bed, appear to be discontinuous, many areas were lined only with the basement membrane between the capillaries and the intercellular spaces of the outer enamel epithelial cells and the capillaries extended deep into the enamel organ in contact with the ameloblasts. These features may be very suitable for carrying large amounts of metabolic materials to the ameloblasts within a limited area. In histological slides, a few capillaries sometimes intruded into the stellate reticulum. Erythrocytes in the capillaries existing in this stellate reticulum have been reported in the rat molar (Liao et al. 1978) but not in other mammals. As described in the present paper, the capillaries do not intrude into the stellate reticulum of the rabbit major incisor, since a double-layered basement membrane is present between the capillaries and the outer enamel epithelial cells.

4. Early maturation zone/stage

The characteristics of the ameloblasts as protein-secreting glandular cells disappear within this zone while the cytoplasmic organelles reveal an atrophic arrangement. Nevertheless, the capillary network and wall are similar in morphology to those in the previous stage. The meshes are elongated to become twice long as those in the previous zone but the fenestrations decrease in number. These features suggest that the ameloblasts no longer require a large amount of metabolic materials.

5. Late maturation and regression zones/stages

The ameloblasts suddenly become atrophic without the pigmentation zone visible in the rat. The papillary cells decrease to one or two layers in a flat arrangement without any papillae. The capillaries with a continuous wall form flat and coarse meshes in contact with the papillary cell layer. The transport of metabolic materials suddenly diminishes at both stages. However, Iwaku et al. (1979) observed in the rat a ladder-like capillary network perpendicular to the tooth axis extending up to the incisal edge in the postsecretion zone (corresponding to the early and late maturation zones of this paper). This difference suggests that the function of the enamel organ may terminate earlier in the rabbit than in the rat, at least when judged from the vascular supply. Since no angiological differences are observed between the RA- and SA-areas, the respective transport of metabolic materials may be similar in amount.

References


Explaination of Figures

Plate I

Fig. 2. Schematic drawing of the whole microvasculature of the enamel organ of the rabbit upper major incisor.

Fig. 3. Whole microvascular cast of the enamel organ of the rabbit upper major incisor. aa: anterior superior alveolar artery.
Plate II

Fig. 4. Schematic drawing of serial changes of the capillary networks during the amelogenesis viewed from the alveolar bone side. Sa: arterioles, sv: venules.

Fig. 5. Whole microvascular cast of the capillary networks of the enamel organ. The upper major incisor was extracted.
Plate III

Figs. 6a and 6b. Frontal sections through the secretion zone.
   6a, a microvascular cast of the rabbit major incisor.
   6b, a histological slide.
Figs. 7a, 7b and 7c. Proliferation zone.
   7a, LM-photograph (toluidine blue staining). ×350.
   7b, SEM-photograph, ladder-like mesh of the capillary network.
   7c, TEM-photograph, continuous-typed capillary.
Figs. 8a, 8b and 8c. Differentiation zone.
   8a, LM-photograph (toluidine blue staining). ×350.
   8b, SEM-photograph, networks composed of continuous-typed capillaries.
   8c, TEM-photograph, continuous capillaries located between ridges of the outer enamel epithelium, inn: inner enamel epithelium,
   oee: outer enamel epithelium, arrows: double basement membrane of the enamel organ and capillaries.
Plate IV

Figs. 9a, 9b and 9c. Secretion zone.
9a, LM-photograph (toluidine blue staining). ×350.
9b, SEM-photograph, polygonal-meshed capillary network.
9c, TEM-photograph, fenestrated capillary. The double basement membrane can be seen.
em: enamel matrix, ab: ameloblast, \( \wedge \) : fenestrations.

Figs. 10a, 10b and 10c. Early maturation zone.
10a, LM-photograph (toluidine blue staining). ×350.
10b, SEM-photograph, oval mesh of the capillary network.
10c, TEM-photograph, fenestrated capillary. The double basement membrane can be seen.
e: enamel, d: dentin, ab: ameloblast, p: papillary cell.

Figs. 11a, 11b and 11c. Late maturation (upper) and regression (lower) zones.
11a, LM-photograph (toluidine blue staining). ×350.
11b, SEM-photograph, coarse oval mesh of capillary networks.
11c, TEM-photograph, continuous capillaries located close to a flattened papillary cell layer.
E: enamel, av: alveolar bone, ab: ameloblast, p: papillary cell.