Tooth Enameloid Formation of a Fish (*Hoplognathus fasciatus*)

—Chronological Relationship of Matrix Formation and Mineralization of the Enameloid and Dentin—

by

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Introduction

The mineralization pattern of enameloid[1] of the tooth of *Hoplognathus fasciatus* has been studied in this department using historadiography[2–4], tetracycline injections[5,6] and other histological methods[7]. The chronological relationship between the commencement of mineralization enameloid and the formation of dentin is histologically clarified in this study.

Materials and Method

Tooth germs removed from the upper and lower jaws of living *Hoplognathus fasciatus* not less than 20 cm long were used in this study. The tooth germs were fixed with Zenker-formalin and prepared without decalcification as paraffin serial sections 5–7 microns in thickness. Serial sections were treated consecutively with hematoxylin and eosin, azan, Heidenhain's iron hematoxylin, Wilder's silver stains and PAS reaction.

Results

Enameloid first forms as a thin layer of homogeneous organic substance that stained with eosin (Fig. 1). This organic matrix of enameloid consisted of blue and dark-brown stained fibrils respectively when azan and silver stains were used. The illustration shows no communication between these enameloid fibrils and the connective tissue fibrils in the dental papillae. However, at the earliest stage an intimate relationship appears to be present between the odontoblasts and the fibrils of the enameloid matrix (Fig. 2).

The thin layer of organic matrix gradually increases its thickness and as the thickness increases, the fibrils increase in diameter. Around the time of completion of future outline of the enameloid organic matrix, these fibrils with large diameters are arranged perpendicular to the surface in the superficial layer of enameloid. In the

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deeper layers, however, they are arranged in various directions (Fig. 3A). These fibrils show collagenous characteristics for they stained blue with azan stain and violet brown with silver stain.

When the future outline of the enameloid matrix is completed, formation of pre-dentin and mineralization of enameloid begins. The newly formed pre-dentin appears to be a continuation of the organic matrix of enameloid (Fig. 3A). One can see a light stained regularly arranged layer of fibrous dentin matrix peripheral to the clearly recognizable odontoblastic layer. The junction between the enameloid and dentin because of its fibrous appearance is rather difficult to detect. A change in stainability, however, occurred in a part of the enameloid adjacent to the pre-dentin indicating the beginning of mineralization. Consequently, the distinction between enameloid and pre-dentin can be readily made at this point. The enameloid portion which stained with hematoxylin is probably where mineralization has started (Fig. 3A). It stained black with Heidenhain's iron hematoxylin stain (Fig. 3B) and reacted positively with PAS (Fig. 3C). On the other hand, azan stain revealed an orange yellow color in the enameloid adjacent to pre-dentin and a blue color of the pre-dentin and most of the remaining enameloid (Fig. 3D). Enameloid which showed orange yellow did not stain with the silver stain (Fig. 3E). However, in this stage, the pre-dentin was negative for PAS reaction and no change in stainability of the pre-dentin occurred with any of the other methods.

Mineralization which started in the enameloid very slowly extended outward over the middle layer. The portion of enameloid in which mineralization was seen in the

Fig. 1. A tooth germ of *Hoplognathus fasciatus* in early stage of development. EO: enamel organ, E: enameloid, OD: odontoblasts, DP: dental papilla, H and E stain.

Fig. 2. Enameloid matrix fibrils and odontoblasts (OD). E: enameloid, DP: dental papilla, Heidenhain's iron hematoxylin stain.

Fig. 3. Stainability of initial mineralized enameloid and predentin. E: enameloid, ME: mineralized enameloid, PD: predentin, OD: odontoblasts.

Fig. 3-A. H and E stain, Enameloid and predentin show pink color and mineralized enameloid blue.

Fig. 3-B Mineralized enameloid shows iron black with Heidenhain's iron hematoxylin.

Fig. 3-C. PAS reaction, Mineralized enameloid shows positive, enameloid and predentin negative.

Fig. 3-D. Enameloid and predentin show blue and mineralized enameloid orange yellow with azan stain.

Fig. 3-E. Mineralized enameloid is hardly stained with silver stain.

Fig. 4. A line (L) densely stained with hematoxylin and hypermineralized enameloid (HE). PD: predentin, ME: mineralized enameloid, H and E stain.

beginning rapidly became hypermineralized. This hypermineralized enameloid portion failed to stain with H and E (Fig. 4). At the same time a line densely stained with hematoxylin appears at the dentinoenameloid junction (Fig. 4). However, this dense line was not observed in all cases.

Following mineralization of the enameloid adjacent to the predentin, mineralization also occurs from the surface of the enameloid and rapidly progresses into the deeper enameloid layer (Fig. 5).

At the time mineralization spread over most of the enameloid, formation of dentin started to occur. Dentin (orthodentin) is formed through mineralization of predentin. Dentin is produced through thickening of the predentin layer previously formed and the dense hematoxylin stained line described above, moves towards the predentin (Fig. 6A). In instances where the dense hematoxylin stained line did not appear, mineralization progressed leaving an unmineralized line stained by eosin in between the mineralized portions of predentin and enameloid (Fig. 6B). This unmineralized line, however, became mineralized soon afterwards. Dentin of these fish teeth as in human dentin matures very early through mineralization of the predentin. Dentin is therefore barely stained by dyes in undecalcified section (Fig. 7).

Discussion

In *Hoplognathus fasciatus*, ten more different stages of its development may be present within the jaws. It has not been decided whether tooth formation in these

Fig. 5. Unstaining enameloid mineralized from both outer (OL) and inner layers (IL). EO: enamel organ, PD: predentin, H and E stain.

Fig. 6. Dentin formation. ME: mineralized enameloid, HE: hypermineralized enameloid, D: dentin, MF: mineralization front of dentin, PD: predentin.

Fig. 6-A. H and E stain.

Fig. 6-B. Unmineralized line (+) between hypermineralized enameloid (HE) and mineralization front of dentin (MF). H and E stain.

Fig. 7. Dentin (orthodentin) shows dentinal tubules. D: dentin, MF: mineralization front of dentin, PD: predentin, H and E stain.

tooth germs of adult fish and tooth formation in the fish fry that shows fewer stages are completely identical. However, based on the unpublished results of observations on tooth germs of *Hoplognathus fasciatus* fry, the difference appears to be insignificant.

KVAM[8,9] showed that the fibers forming the organic matrix of enameloid of fish teeth were of mesodermal origin. Others[7, 10–14] have observed collagen fibers forming the organic matrix of enameloid of *Hoplognathus fasciatus* and in the teeth of several other osseous fishes. Moreover, these fibers appeared to be intimately related with odontoblasts. Although no confirmation is available, these collagen fibers are probably connected with collagen fibers of the dental papilla. Since the borderline between predentin and enameloid is barely distinguishable because of mineralization, an intimate relationship between these matrix fibers of both tissues is suggested.

The directional arrangement of fibers in enameloid corresponded with the descriptions given by ISOKAWA and his colleagues with respect to those seen in porgy[10, 11], file-fish[12] and *Samicossayphus*[13,14].

The mineralization of enameloid was examined in the present study by the reaction to different stains based on studies by OKADA et al.[15], YAMADA[16] and SUGA[17,18]. The mineralization pattern of enameloid has also been studied by TSUBO-UCHI[2], ISOKAWA[3], ISOKAWA et al.[4], HASEGAWA[5], TODA et al.[6] and MISU[7]. However, only a few assumption[2,7,12] have been made regarding the beginning of mineralization of enameloid and that of matrix formation of dentin.

The present study verified the fact that, at the dentinoenameloid junction, the organic matrices of enameloid and dentin are so intimately bound together that they are almost indistinguishable. However, the junction of these two substances may be distinguished through the mineralization of enameloid. This finding should provide strong support for the studies of KVAM[9] who pointed out the mesodermal origin of dental enameloid of fish. Furthermore, no difference seems to be present in fibers constituting the organic matrices of enameloid and dentin. Though the present study fails to provide sufficient material, the morphological difference between enameloid and dentin could be a difference in the ground substance between the matrix fibers and the organic substance deposited there.

The present study has confirmed previous studies already reported by this department to the effect that mineralization of enameloid begins on its dentinal side and that it hypermineralizes rapidly but that the spread of the mineralization over the surface is very slow.

At the beginning stage of mineralization of the enameloid, the formation of dentin has started as a thin layer of predentin formed on the surface of the dental papilla. This follows the formation of the enameloid organic matrix. However, until there is considerable mineralization of enameloid the first formed predentin fails to further increase its thickness and to mineralize.

When the mineralization of enameloid has made considerable progress, continued formation of dentin is noted. The predentin layer is thickened and inorganic substances are deposited beginning in the portion adjacent to the enameloid.

Dentin of this fish matures along with the deposition of inorganic material as does the dentin of man and other mammals. Since the dentin of *Hoplognathus fas-*

ciatus is orthodontin, dentinal tubules are found in the dentin and these indistinct dentinal tubules may be observed in H and E stained sections.

Conclusion

The chronological relationship between jaw tooth enameloid, dentin formation and mineralization of the developing tooth germs for more than ten generations of *Hoplognathus fasciatus* was studied histologically. The following are the conclusions derived from the study:

- (1) Fibers of the organic matrix of the enameloid of the fish teeth are examined
- (2) The matrix fibers of enameloid and dentin blend with each other and the former were barely distinguishable from the latter because of a change in stainability due to early mineralization.
- (3) The mineralization of enameloid starts at the portion adjacent to the dental papilla after the outline of the organic matrix of enameloid has been completed. However, completion of the mineralization of the enameloid matrix subsequently takes place from the surface of enameloid.
- (4) Before the beginning of first mineralization of enameloid, dentin appears as a thin layer of predentin. However, formation of dentin does not progress further, until mineralization of enameloid has made considerable progress.

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