Fine Structure of Susceptible Enamel Lamellae against Dentin Caries in the Fissures of Rat Molar Teeth

Tetsuo KODAKA, Masayuki ABE and Shohei HIGASHI

Second Department of Oral Anatomy, Showa University School of Dentistry
1-5-8 Hatanodai, Shinagawa-ku, Tokyo, 142 Japan
(Chief: Prof. Shohei Higashi)

Abstract: It has been reported that the fissures of rat molar teeth are apt to cause dental caries. In this study, we observed enamel lamellae in the fissures of caries-free molar teeth of rats, 2 and 4 weeks after birth, by transmitted light microscopy with decalcified sections and by scanning electron microscopy with EDTA-treated samples. Enamel lamellae were always present buccolingually in the fissure enamel. In unerupted teeth, the organic lamellae showing an intermittent structure were composed of the organic matrix, derived from ameloblasts and reduced enamel epithelium, occasionally with these epithelial cells. After eruption, the thin organic membrane of enamel lamellae was probably derived from saliva as well as the organic matrix formed during the enamel formation. The lamellae also contained oral microorganisms and fibrous structures, some of which were formed from microorganisms, under the widely opened fissures containing the deposits of oral microorganisms and diet remnants. Thus, we strongly suggest that such fissure lamellae of rat molar teeth are apt to become the passing point in the way to inducing the dentin caries.

Key words: rat molar teeth, fissure enamel lamella, fine structure, dentin caries.

The fissure enamel of rat molar teeth is apt to cause dentin caries as well as enamel caries. The decay of the fissure enamel will succeed to the remarkable dentin caries. In caries-free molar teeth of rats, the fissure enamel shows hypocalcification. Such a calcification may induce enamel caries. In addition, the porous structure of the prisms and enamel lamellae will become the passing point in the way to inducing the dentin caries. However, the lamella structures in the fissure enamel of rat molar teeth have not been clearly elucidated except for our recent study, although Rosebury et al. reported organic lamellae in the decalcified sections of rat molar teeth possessing dentin caries in the fissures. In this study, we observed the fine structure of fissure lamellae in caries-free molar teeth of rats by transmitted light microscopy (LM) and by scanning electron microscopy (SEM).

Materials and Methods

Five Sprague-Dawley (SD) rats at 4 weeks after birth and five SD rats at 2 weeks after birth were killed, and the mandibles were fixed in 10% buffered formaldehyde at pH 7.2. In the mandibles of 4-weeks rats, caries-free first and second molar teeth were erupted, but no teeth were erupted in those of 2-week rats.

Half of right or left mandibles of 4-week and 2-week rats were used for SEM observations. The samples of 4-week rats were ground mesiodistally with grinding stones. The samples of 2-week rats were fractured mesiodistally after embedding with
styrene resin, and then the resin were dissolved with propylene oxide\textsuperscript{13}. These SEM specimens were treated with 2.5% ethylenediamine tetra-acetic acid (EDTA) for 1 to 4 h and rinsed under running tap-water. This was followed by dehydrating with ethanol and drying at the critical point of CO\textsubscript{2}.

Figure 1. Scanning electron micrograph (SEM) of the lingual enamel in the mandibular first molar tooth of a 4-week rat. EDTA treatment after brushing with toothbrush. F: fissure. L (arrow): enamel lamella. E: etched enamel surface. Bar = 50 \textmu m.

They were observed with a Hitachi S-430 SEM at 15 kV after coating with a platinum-palladium layer of about 15-nm thickness. The remaining mandibles of 4-week and 2-week rats were used for LM observations. The samples were decalcified with 10% EDTA at pH 7.2 and embedded with paraffin or cellulose nitrate (celloidin). The serial mesiodistal sections were stained with hematoxylin and eosin (H & E) or van Gieson’s stain with hematoxylin post-staining. These stained sections were photographed with a Nikon LM.

In a recent study, we reported the distribution pattern of enamel lamellae in rat molar teeth. These samples were also used for the SEM observations of fissure lamellae in this study.

Results

Figure 1 shows a SEM image of the lingual enamel surface in a first molar tooth of a 4-week rat. The lamella invading the fissure buccolingually was observed. Figures 2 to 4 show LM images of the fissure in the decalcified mesiodistal sections of 4-week rat molar teeth. In Figure 2, the lingual-side surface of the first molar tooth was cut tangentially. The organic lamella extended from the fissure floor containing deposits to the attachmental epithelium of gingiva. The sections of Figures 3 and 4 were cut mesiodistally through the fissure floor zones of molar teeth. The lamellae invaded the enamel from the fissure floors towards the dentin surface. In Figure 3, the lamella arised from the dentin surface, although it was not connected with the lamella from the fissure floor. The break might be caused by the sample procedure. The deposits in the fissures were occupied mainly with diet remnants.

Figures 5 and 6 show SEM images of the mesiodistal ground sections of molar teeth treated with EDTA in 4-week rats. The organic lamellae extended from the fissure floors to the dentin surfaces. In Figure 6, fine slits and pits were observed on the dentin surface. They might have been connected with the lamella.

Figures 7 to 10 show fine structures of the organic lamellae under the SEM observations. The enamel lamellae, which succeed to the organic membrane on the fissure floors containing the deposits of oral microorganisms and other materials (Figure 7), consisted of thin membranous

Figures 7 to 10. SEMs of the enamel lamellae of 4-week rat molar teeth. EDTA treatment. L: enamel lamella. M (arrowhead): microorganism. F: fissure floor viewed from the dissolved-enamel side. FD: fissure deposits. E: etched enamel surface. Bars= 5 μm (7, 8, 10), 0.5 μm (9).

structures (Figures 7, 8) and fibrous structures (Figures 7, 9, 10). Oral microorganisms were frequently observed (Figures 7, 9, 10). In Figure 10, the fibrous structures were formed from oral microorganisms.

In unerupted molar teeth of 2-week rats, the ameloblasts in the fissure floors were occasionally changed. Figures 11 to 13 show LM images of the fissures in the decalcified mesiodistal sections. During the developing stage of enamel formation,
several nuclei of ameloblasts were present in the mesial-side of the ameloblast layer (Figure 11). After absorption of the organic matrix in the enamel formation, several ameloblasts or reduced enamel epithelium invaded the enamel matrix (Figure 12). Lamellae, which were similar to the enamel lamellae in erupted teeth (Figures 2-4), were frequently observed (Figure 13).

When observed the ameloblast layer in the fissure floors by SEM after EDTA treatment, fine organic lamellae were frequently found (Figures 14, 15). The lamella structures were not continuous but intermittent (Figure 14). The lamella succeeded to the basal lamina of the ameloblast layer (Figure 15).

In erupted teeth of 4-week rats, as shown in Figure 2, enamel lamellae containing epithelial cells with a nucleus were occasionally observed in the decalcified sections (Figures 16, 17), although the lamella in Figure 17 was not the fissure lamella.

Discussion

In human teeth, enamel lamellae are composed of thin organic membrane in three-dimensional structure\(^{15}\), and some lamellae connected with the dentinal tubules\(^{16,17}\). The organic matrix containing primary enamel cuticle are derived from ameloblasts and reduced enamel epithelium during the developing to maturation stages of enamel formation\(^{17-19}\). After eruption, oral microorganisms invading the lamellae may cause dentin caries in the fissures\(^{20}\).

In rat molar teeth, some lamellae invaded the

dentin (Figure 6), which may be connected with the dental tubules, as recently reported. In the fissures, the enamel lamellae were always present (Figures 1-6) as recently reported. In this study, we revealed that the structure of enamel lamellae was similar to that in human teeth. That is, the fissure lamellae, are composed of the organic matrix containing primary enamel cuticle, derived from ameloblasts and reduced enamel epithelium, occasionally with these epithelial cells in unerupted molar teeth (Figures 11-15) and erupted molar teeth (Figures 16, 17). However, in erupted teeth, the fissure lamellae in spite of caries-free teeth frequently contained oral microorganisms and fibrous structures as well as thin organic membrane under the fissure deposits including numerous oral microorganisms (Figures 7-9). The membrane will contain secondary pellicle derived from saliva (secondary enamel cuticle) as well as primary cuticle. On the other hand, some fibrous structures were formed from oral microorganisms.

When the microorganisms in the fissure lamellae are caries bacteria, their bacteria will easily reach the dentin under the fissures throughout the fissure lamellae. Thus, we suggest that such fissure enamel lamellae in rat molar teeth are apt to become the passing point in the way to inducing the dentin caries.

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