# Detection of Tooth Enamel Microstructural Transformations during De- and Re-mineralization in the Early Stage of Caries

Dental caries is one of the most common diseases in the world. In the aged society of Japan, preventing the loss of teeth by caries and periodontal disease is considered as a national health issue by the Ministry of Health, Labour and Welfare. Because the loss of teeth causes the insufficient chewing of food, caries threatens not only oral but also systemic health and decreases the quality of social life. Moreover, conserving healthy teeth is important to enjoy daily life with healthy eating, talking and smiling. Hence, preventing caries is important to live a long and healthy life. Since clinical efforts alone cannot eradicate caries, individual daily prevention efforts are important to conserve healthy teeth.

The development of dental caries is regarded as a chronic process that typically occurs over a period of several years. A tooth is covered with enamel, most of which consists of needle-shaped crystals of hydroxyapatite (HAp) that contain calcium and phosphate as the main component (Fig. 1). The acidogenic microorganisms ferment ingested carbohydrates and develop a dental plaque with a variety of organic acids excreted as metabolic by-products. These acids dissolve the subsurface enamel beneath the plaque (demineralization), while the surface of the enamel is conserved. At this early stage of caries, the demineralized enamel area is called a subsurface lesion, and is observed clinically as a white opaque spot. In such a stage, the subsurface lesion can be reversibly cured (remineralized) to a nearly sound state without surgical procedures [1].

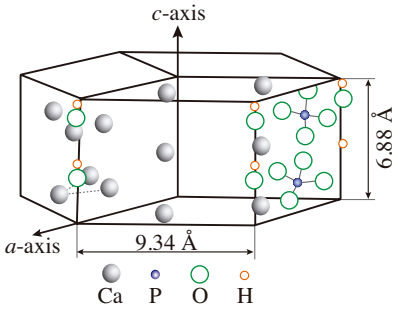


Fig. 1. Detailed crystal structure of hydroxyapatite.

In usual life, remineralization occurs between meals by salivary components such as calcium and phosphate. However, occasionally, the remineralization cannot overcome the demineralization, and the caries lesion gradually develops to irreversible stages. This process has been studied extensively by transversal microradiography (TMR) [2]. TMR provides the mineral distribution in the enamel at a micrometer order to a plano-parallel direction, and used as the gold-standard method for the analysis of demineralization and remineralization in dental science (Fig. 2). However, TMR cannot provide evidence on the distribution of HAp crystallites [2,3]. In fact, whether the restored subsurface is filled with HAp crystallites as in the sound enamel or with other forms of calcium-phosphate salts (e.g., octacalcium phosphate, amorphous calcium phosphate) cannot be determined by TMR.

We developed a new method of determining the

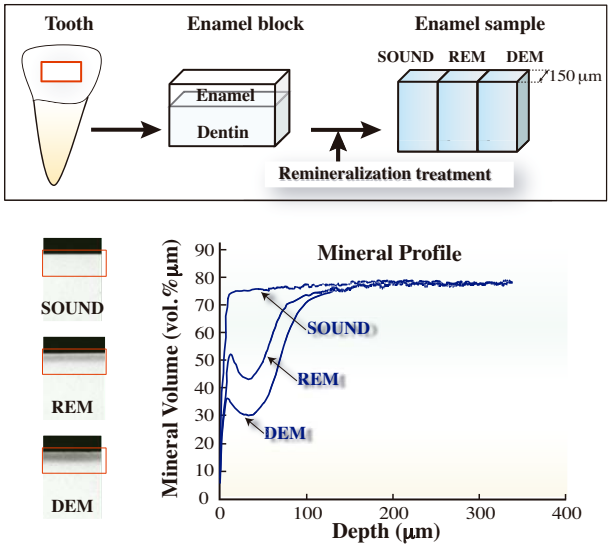


Fig. 2. Preparation of tooth enamel sample. Typical mineral distributions obtained by the transversal microradiography of tooth enamel after demineralization (DEM) and remineralization (REM) in comparison with the untreated enamel (SOUND).

HAp crystal content profile of the 200 μm subsurface area. X-ray diffraction is used to measure the local quantity of HAp crystals. Thin tooth sections containing sound and demineralized enamels were prepared and analyzed at beamline **BL40XU**. A 6 μm X-ray microbeam was produced with a pinhole. The distance from the sample to the detector was 150 mm for the wide-angle X-ray diffraction (WAXRD) and 3000 mm for the small-angle X-ray scattering (SAXS). The sample was moved at 5 μm step so that the X-ray beam scanned it from the surface to the base, and the WAXRD and SAXS images were simultaneously obtained at each position (Fig. 3). From the WAXRD image, the intensity of the arc corresponding to the diffraction from the (100) plane of HAp was integrated and plotted against the depth of the enamel to produce a HAp profile. As shown in Fig. 4, the intensity was decreased by the demineralization treatment, and the profile was essentially the same as that obtained by the TMR method. Moreover, because, apart from its intensity, the diffraction pattern was unchanged by demineralization treatment, the HAp crystallite structure is conserved in the demineralized enamel. The SAXS intensity represented the amount of voids in the enamel crystallites. It increased in the subsurface area where demineralization was observed in WAXRD and TMR, showing that the crystallites became thinner and voids were created between them. These results suggest that the size of the HAp crystallites is decreased by demineralization but their orientation is conserved [4]. This technique is expected to be useful for analyzing the demineralization and remineralization mechanisms of tooth enamel.

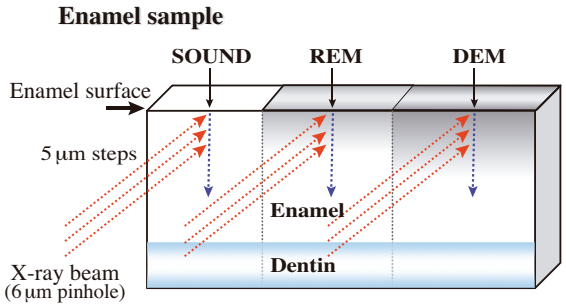


Fig. 3. Scheme of X-ray diffraction analysis of enamel samples.

It is anticipated that, if calcium is positively provided by the ingestion of calcium-rich foods under neutral condition, the calcium ions from foods and phosphate ions from saliva can facilitate the remineralization of the early caries [5]. We found that phosphoryl oligosaccharide of calcium (POs-Ca) provides "active" calcium ions to the subsurface lesion under neutral pH condition. It is soluble in saliva without forming precipitates with phosphate or carbonate ions and drastically promotes the remineralization [5]. In our latest study, we found that the remineralization with POs-Ca was actually "recrystallization": the regrown HAp crystallites were found by microbeam X-ray diffraction to have the same orientation as the original sound enamel. This demonstrates that POs-Ca can heal the lesion through the recrystallization of HAp crystallites in the enamel with regard to both volume and orientation.

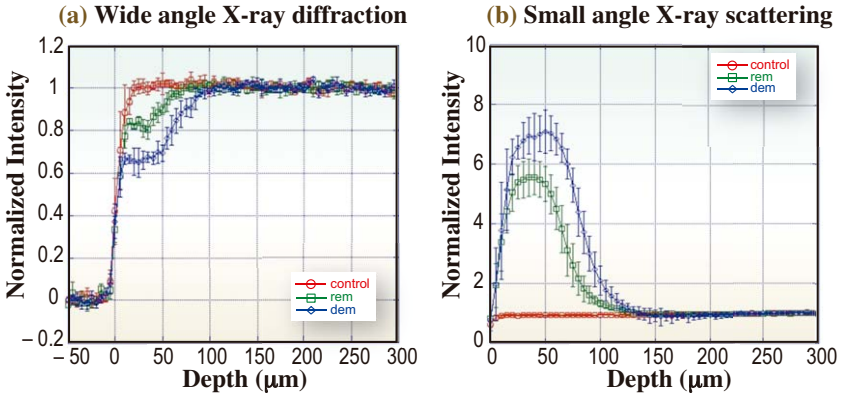


Fig. 4. Plots of X-ray diffraction intensities against depth of sound (control), remineralized zone (rem), and demineralized zone (dem) enamels. **(a)** Wide-angle X-ray diffraction. **(b)** Small-angle X-ray scattering.

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