Microarchitectural Study of Rodent Enamel by X-ray Microtomography

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Introduction

Rodent dental tissue has a complex, textile-like microstructure consisting of many regularly interleaved enamel rods. We have been studying the relationship between the dental microstructure and the resulting mechanical properties. One of the proteins that influences the biomineralization process is amelogenin, which makes up more than 95% of the proteins during the nucleation stage. Knowing the function of this protein in the biomineralization process and its effect on the final enamel microstructure will provide important clues toward gaining a fundamental understanding of enamel formation.

We have found that there is a strong dependence on the details of the microstructure, as modified by altered amelogenin protein through transgenic mice. However, there are no 3-D studies of the exact textile weave of the enamel rods in any dental tissue, let alone a 3-D study of our novel samples. Therefore, the purpose of this research is to study the genetically altered amelogenin protein on the microarchitecture of the enamel rods by using high-resolution x-ray microtomography. This study will allow us to gain a greater understanding of the relationship between the form and function of the enamel rod textile and the breakdown of this relationship as a consequence of altered amelogenin protein. The overall study is aimed at understanding the role of amelogenin protein in enamel regeneration, and it constitutes one step toward the long-term goal of clinical regeneration of enamel in human teeth. Such a process would have wide-ranging health benefits.

Methods and Materials

Three lines of mouse teeth were studied: a normal (control) line and two lines grown with altered amelogenin proteins designated as A-domain and B-domain deletions. Details on establishing these mouse lines are found in References 1 and 2. For the x-ray microtomographic measurements, mature sections of upper incisors were harvested from euthanized animals and sterilized. They were then cut to $0.2 \times 0.2 \times 1.5$ mm. Beamline ID-20 at the Pacific Northwest Consortium Collaborative Access Team (PNC-CAT) sector at the APS, operating at approximately 11 keV, was used to obtain tomographic data. The experimental setup was tuned to a resolution of 1-μm^3 voxel. Image processing and reconstructions were handled by programs developed in IDL.

Results

While image processing is ongoing, we have obtained results that are beginning to resolve the enamel rods that are approximately 3-μm wide. Shown in Fig. 1 is a slice of the normal incisor that clearly shows the enamel and dentin tissues. Near the dentin-enamel interface, enamel rods reveal themselves mostly as parallel lines oriented roughly 60° with respect to the dentin-enamel interface.

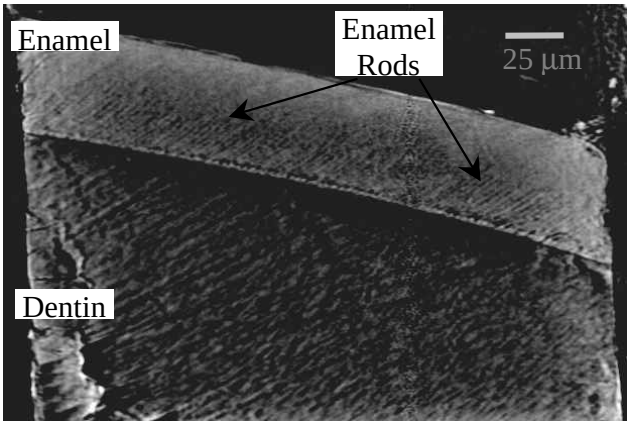

FIG. 1. Example slice of the normal mouse tooth revealing enamel rods in the enamel region.

Discussion

The microstructure of enamel in the mouse incisor consists of two sets of intermeshed bundles of hydroxyapatite crystals. These bundles, also termed enamel rods, make up 99% of the enamel tissue. The high degree of mineralization and this unique microstructure provide the necessary strength to perform dentition functions. Our aim in this work is to resolve this intermeshed structure in 3-D space in the normal and transgenic samples. Such a study will allow us to determine the effect of altered amelogenin on the 3-D microstructure. Our current effort focuses on further improving the resolution to clearly reveal the intermesh network through filtering techniques and choosing

optimal display angles. Doing so will allow us to quantify the 3-D network and compare the normal microarchitecture with that grown with altered amelogenin.

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