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MMP20-ablated Induced Aberrant Mineralization in Early Secretory Enamel

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Matrix metalloproteinase-20 (MMP20) is a kind of proteinase expressed in tooth development. During the development of teeth, the enamel matrix proteins (EMPs), which are the essential components for the formation of the enamel layer, are secreted by ameloblasts in the secretory stage [1-3]. Following the expression of EMPs, MMP20 is secreted to cleave EMPs into fragments, which themselves are essential components required for proper enamel formation [4, 5]. MMP20 mutations cause the amelogenesis imperfecta, a group of inherited disorders characterized by abnormal enamel formation [6]. The investigation of MMP20 could not only provide a different insight into the therapy of amelogenesis imperfecta, but also offer a new aspect for artificial teeth.

Yamazaki et al. reported that although the enamel in MMP20-null (KO) mice went through a similar growth pattern as those from the wild-type (WT) mice in the initial formation process, plate-like crystals appeared randomly and gradually dominated the enamel [4]. Additionally, Bartlett et al. have shown that a similar structure in the KO enamel was observed and identified as octacalcium phosphate which is different from the components in the WT enamel, which are majorly composed of hydroxyapatite [5]. In this research, the effect and mechanism of MMP20 were investigated by observing the WT enamel and KO enamel in the early secretory stage. In the transmission electron microscope (TEM), bright field (BF) imaging, selected area diffraction (SAD), and electron energy-loss spectroscopy (EELS) were used to characterize the components. The influence of MMP20 on the crystallization process of enamel will be discussed in this contribution.

The aberrant mineralization induced by the loss of MMP 20 was evaluated with the BF images and the SAD patterns (Figure 1). In the WT enamel, the enamel rods are well-aligned and follow the retracting direction of the ameloblast, and the scallop-shaped dentin-enamel junction (DEJ) can be observed clearly. In contrast to the WT enamel, plate-like crystals and dark blocks appeared aberrantly in the KO enamel (Figure 1E), and small dark spots and fibers spread in the KO enamel matrix (Figure 1F). Plate-like crystals are darker than the surrounding matrix, and there are lots of small pores distributed inside randomly. The diffraction pattern inserted in Figure 1E showed a different feature compared to the WT enamel. The diffraction pattern of KO crystals is more similar to octacalcium phosphate, and the one from WT enamel is close to the crystal structure of hydroxyapatite.

EELS was used to identify the components in both WT and KO enamel. The energy loss near-edge structure (ELNES) of P-$L_{2,3}$, Ca-$L_{2,3}$, C-K, and O-K edges were studied from the WT enamel crystal, the space of Weber, the plate-like crystals, and the KO enamel matrix (Figure 2). In the P-$L_{2,3}$, Ca-$L_{2,3}$ edge, except for the spectrum from the spaces of Weber, all the spectra were similar, indicating the lack of phosphorus and calcium intensities in the spaces of Weber. For the C-K and O-K edges, some differences could be observed. For example, peak A labeled in Figure 2C was only observed in the WT enamel. However, the spectrum of reference materials, such as hydroxyapatite, octacalcium phosphate, tricalcium phosphate, and type-I collagen, are needed to provide a comprehensive analysis of the materials.

In this research, the aberrant enamel formation induced by the lack of MMP20 was observed. The plate-like, porous enamel crystals emerged irregularly, and numerous dark spots appeared in the amorphous enamel matrix. The EELS analysis revealed some major peaks in P-$L_{2,3}$, Ca-$L_{2,3}$, C-K, and O-K edge. However, the EELS data of reference materials and further identification of the corresponding peaks still needed to be achieved in the future.

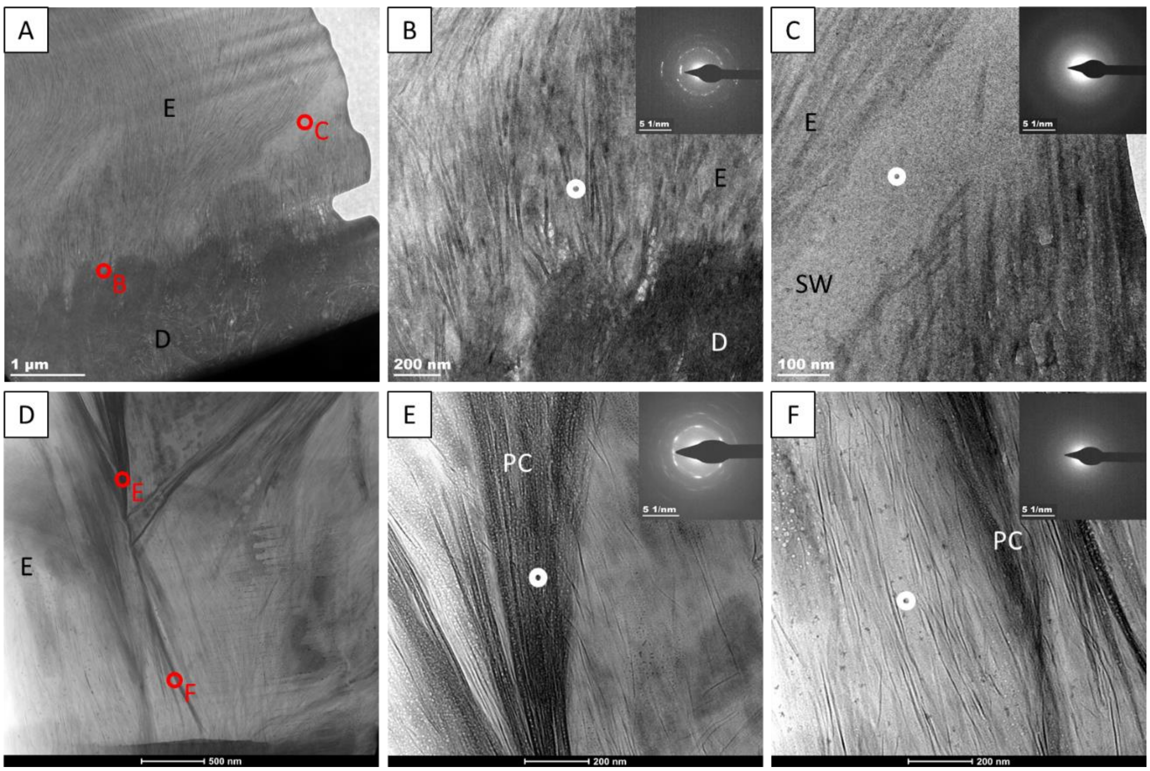

Figure 1. BFTEM images from WT (A, B, and C) and KO (D, E, and F) mice. The white circle indicated the diffraction pattern inserted in the images. E, enamel; D, dentin, SW, spaces of Weber; PC, plate-like crystal.

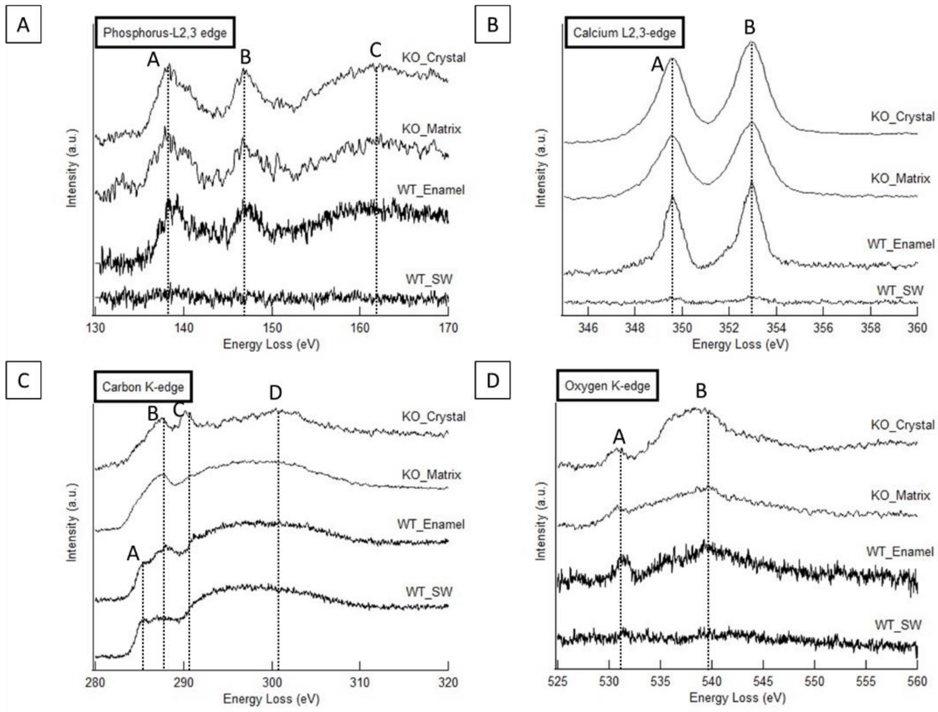

Figure 2. (A) Phosphorus-L2,3, (B)Calcium-L2,3, (C)Carbon-K, and (D)Oxygen-K ELNES from WT enamel crystals (WT_Enamel), spaces of Weber from WT mice (WT_SW), KO plate-like crystals (KO_Crystal), and KO enamel matrix (KO_Matrix).

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