

Transmission Electron Microscopy Studies of Calcium Phosphate Biomineralization

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The continuous demineralization and remineralization processes continuously happen on the dental enamel. Once the balance between these two processes is broken, dental erosion or dentine hypersensitivity will happen. Therefore, elucidation of the biomineralization pathway *in vitro* will help dentists to find out a more precise strategy to maintain the oral health. In fact, the biomineralization of apatite, the core building block of the enamel, has been studied for decades, but due to resolution limit in the imaging, sub-micron level details of this process are still not clear. There are still several important questions to be answered: 1) How do the Ca^{2+} and PO_4^{3-} ions exist in aqueous solution, as ions or as metastable clusters, like Posner cluster ($\text{Ca}_9(\text{PO}_4)_6$), the smallest unit in calcium phosphate? Posner and Betts [1] 2) How are these clusters or ions combined, as single ions attachment or as cluster aggregation? 3) How does the amorphous calcium phosphate (ACP) form the crystal structure [2], *via* direct phase transformation or *via* dissolution and recrystallization processes? Dynamical high-resolution imaging is needed to be carried out for the investigation of the apatite mineralization pathway, so that we can answer these questions. Fortunately, in recent years, liquid cell (Scanning) Transmission Electron Microscopy ((S)TEM) has enabled the investigation of such dynamic biological processes in sub-micron scale due to the Z contrast. In this research, the liquid cell STEM imaging was used to investigate the biomineralization process. A mineral solution was encapsulated in liquid cell, and then it was imaged *via* STEM. Particles with 10-15 nm sizes nucleated from the solution and then attached onto each other to form a larger loosely bound cluster as shown in Figure 1 (b). The whole process was captured dynamically. Electron Energy Loss Spectroscopy (EELS) and Selected Area Electron Diffraction (SAED) were carried out to identify the chemical composition and crystal structure of the newly formed crystals, respectively. EELS confirmed the existence of calcium ions. Our results partially disagreed the novel pathway for the crystallization of calcium phosphate, which insisted crystalline phase calcium phosphate formed by certain stages: metastable ion clusters, amorphous phase, and then the amorphous transform to crystal. From our research, we proposed a new pathway for the mineralization process, first some metastable cluster formed, because of the local fluctuation of ions, and then based on some driven force (interface energy), these metastable clusters formed some smaller crystal particles (10-15 nm) and finally these small particles aggregated to form crystal phase in a larger size (around hundreds nanometers). Moreover, for this test, no trace of existence of amorphous phase was observed, which dissented the proposed by other researchers [2, 3].

References:

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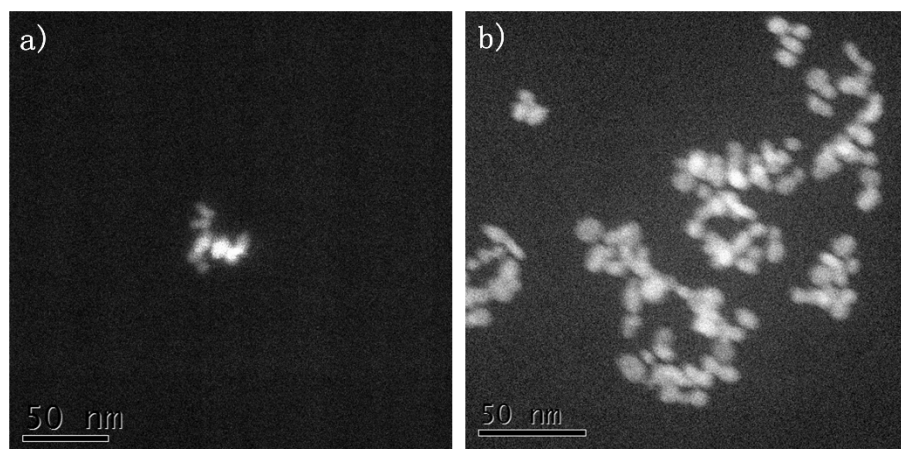


Figure 1. STEM images of newly formed calcium phosphate particles (a) and the aggregated particles cluster (b) in the liquid.