Biological Hydroxyapatite Structure Determination by Electron Crystallography Techniques

Abdulelah S. Alrebaish[1,2] Otto C. Wilson Jr.[1]

[1]Department of Biomedical Engineering, The Catholic University of America, Washington D.C., USA

[2] Department of Biomedical Technology. King Saud University, Riyadh, Saudi Arabia

Statement of purpose: The biomimicry of the structural features and crystallinity of biological hydroxyapatite (BHAp) is important for the fabrication of advanced hydroxyapatite (HAp) biomaterials for various applications in medicine. Two distinctive features of the bone apatite at the nanoscale are important. First, plate like BHAp crystals are crosslinked with collagen fibrils. Secondly, the observation of primary particle aggregates which provides evidence of aggregative growth mechanisms. These primary particles are believed to be amorphous calcium phosphate based on diffraction techniques. However, these techniques are inconclusive since small particle size particles give broad diffraction peaks which result in ring patterns akin to amorphous materials. Here we study the crystallinity and orientation of the primary building units of BHAp using high resolution transmission electron microscopy (HRTEM) by image processing and simulation (S-HRTEM).

Methods: The data analyzed in this study was produced from an investigation on the ectopic biomineralization of BHA in a rat model. HRTEMs were analyzed by Fourier transform direct methods to study the crystallographic orientation of the nanoparticles. S-HRTEM was used to assess the crystallinity of the primary particles and to compare the thickness of the particles. The S-HRTEM was done by applying the multi-slice method. Fourier transform image processing (Geometric phase analysis) was performed on more than 100 particles to measure the interplanar spacing. This enabled the calculation of lattice parameters of the primary particles.

Results: The primary particles showed single crystal characteristics by the FFT diffractograms. The measured Bragg's spots from the diffractograms are equivalent to the (3 1 0), (2 0 2), and (-1 -1 2) interatomic planes of HAp. Figure 1 shows the orientation of the particles where chain-like behavior and attachment are observed over the (3 1 0) and (-1 -1 2) planes. Most particles show a common crystallographic orientation. However, a few particles embedded within the aggregates are randomly oriented. This random orientation observed along with small crystal size explains the ring-like features in the diffraction patterns of BHAp primary particles. To assess the crystallinity, the primary particles were compared with the S-HRTEM images of HAp. The best resemblance was found around 86.4 mm defocus and in less than 2 nm thickness as seen in Figure 2, a. The S-HRTEM image of HAp was compared with a particle from Fig.1. as can be seen in Fig 2, b. The spot pattern shows high resemblance with HAp oriented along the [-1 -3 1] zone axis. Other areas within Fig.1 show less contrast which can be indicative of thickness variation, or crystal tilt. The S-HRTEM of HAp reveals the crystalline nature of the primary HAp particles. The (3 1 0) and (2 0 2) planes in the diffractograms were masked and the inverse Fourier transform was applied for over 100 particles. The spacing of 641 lattice fringes were measured for each direction. The result shows the d-spacing of (3 1 0) is about 0.2260 +/- 0.01 nm, and for the (2 0 2) is about 0.2631 +/- 0.01 nm. The a and c lattice parameters for particles with hexagonal structure were calculated based on the d- spacing equation of such structures. The findings reveal that the lattice parameter in a and b directions is 0.9409 nm while in the c direction is 0.6887 nm. When these values are. compared with the lattice parameters of HAp (a=b= 0.9432 nm and c= 0.6881 nm) we find that there is a reduction in the a and b directions and an increment in the c direction of BHAp crystals. Such phenomena occur when carbonate ions substitute for phosphate ions in the HAp lattice (type B Carbonate HAp).

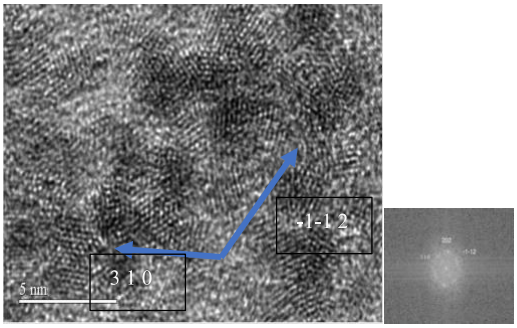

Fig. 1. BHAp primary particles with diffractograms in the subset. Arrows show the orientation of the (-1-1 2) and (3 1 0) planes.

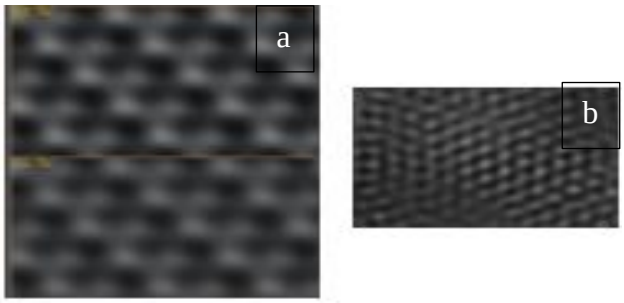

Fig.2. (a) shows the SHRTEM of HAp at 86.4 mm defocus for 2 and 1.5 nm thicknesses. (b) a particle cropped from Fig. 1.

Conclusion: The findings of this study reveal that the primary building units of BHAp are homogenously nanocrystalline BHAp in nature and aggregate with a common orientation. The lattice parameters show they are carbonate substitute HAp. Such findings are important in inspiring the tailoring of next-generation HAp biomaterials for various biomedical applications.