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Characterization of Original Bioapatite and Nano-Scale Heterogeneities in Fossil Bone with Transmission and Scanning Electron Microscopy

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Bone is a complex hierarchical structure composed of up to 98 weight % bioapatite ($Ca_{10}(PO_4)_6(OH)_2$) nano-plates carefully deposited onto a nano-rope matrix of collagen protein. Bone is an intriguing composite from the perspective of biomimicry: the toughness of bone far exceeds that of the brittle bioapatite phase alone. Factors like size, purity, and location of the nano-plates all contribute to the impressive mechanical properties of bone, but quantitative mechanical performance investigations have been largely restricted to fresh, modern mammalian and avian tissues [1]. Current attempts to fabricate biomimetic bone for advanced materials applications are all based on an inherently limited understanding of the "structure of bone." In fact, the femur of a 100-ton *Brachiosaurus* may well be the superior analogy, versus human bone, for a fatigue-resistant structural material. To date, however, there has been no systematic investigation of the hierarchical bone structure of any extinct species. Thus, despite the potential wealth of precise evolution-driven structural information held in the bones of megafauna like *Brachiosaurus*, we have no demonstrated methods for characterizing the nano-scale structures of these amazing natural materials. *Why?*

The first applications of electron microscopy to fossil bone were in the 1960s [2]. Decades later, only a handful of papers on the application of TEM to fossils can be found in the literature [3] [4]. The paleontology community has largely focused on the texture analysis of fossil bone, documenting nano-plate size and relative location/orientation with indirect X-ray methods [5]. Based on this type of data, there exists a common opinion that the bioapatite nano-plates coarsen substantially during fossilization. It should be noted, however, that even the modern synchrotron beam with a spot size of 50 μm still samples over 10^6 nano-plates simultaneously. Similarly, investigation of fossil bone composition with SEM energy-dispersive X-ray spectroscopy (EDS) also lacks sufficient spatial resolution to clearly document the nano-scale heterogeneities associated with the fossilization process.

Our study had two main goals. The first was to conclusively demonstrate the presence of original bioapatite in fossil bone with a combination of standard TEM techniques. The second goal was to elucidate the presence of nano-scale structural and compositional heterogeneities in fossil bone, with direct implications for all future investigations of fossil bone texture analysis and micron-level compositional investigations. Identification of nano-scale heterogeneities was achieved with a combination of SEM and TEM. The specimen set included both fossil and modern bones. SEM specimens were small, polished sections removed from the bulk bone. TEM specimens included both finely ground fracture specimens and ion-milled thin sections, preserving the original orientation of the bioapatite nano-plate with respect to the bulk bone tissue. We believe that our TEM data conclusively demonstrates the exceptional preservation of original bioapatite in multi-million-year-old fossil bones. We also document specific categories of nano-scale structural and compositional alteration of fossil bone, including the effects of bioerosion and geological aqueous processes [6].

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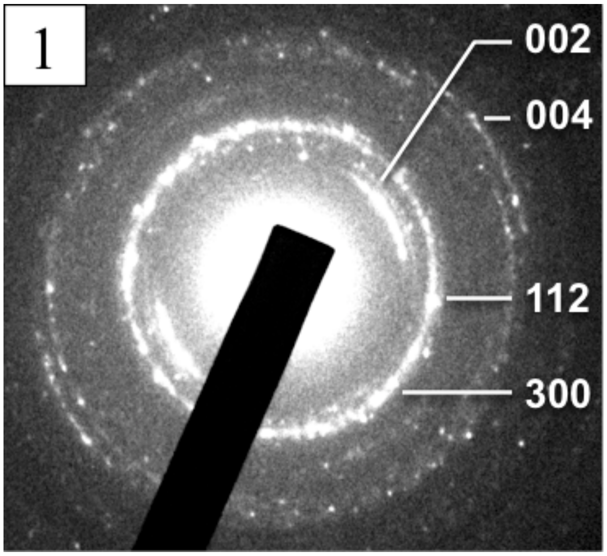

FIG. 1. Indexed diffraction pattern of fossil bioapatite with partial rings due to preferential orientation. Region image is inset **a,** FIG. 2.

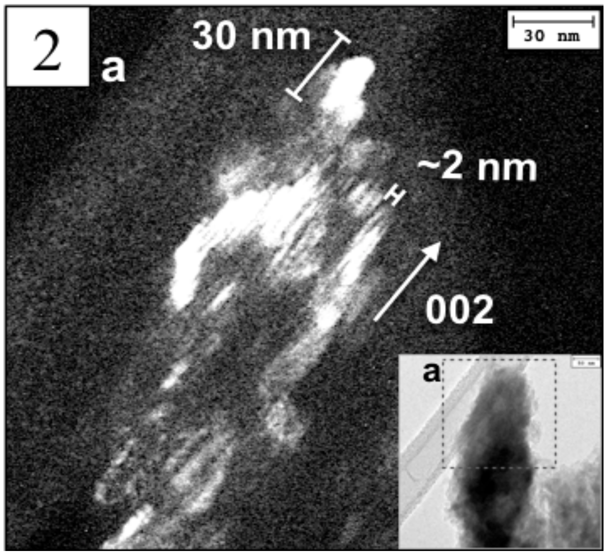

FIG. 2. Dark-field image of fossil mineralized microfibril. Nano-plate dimensions and orientation indicated. Inset of bright-field image with region of interest, **a**.

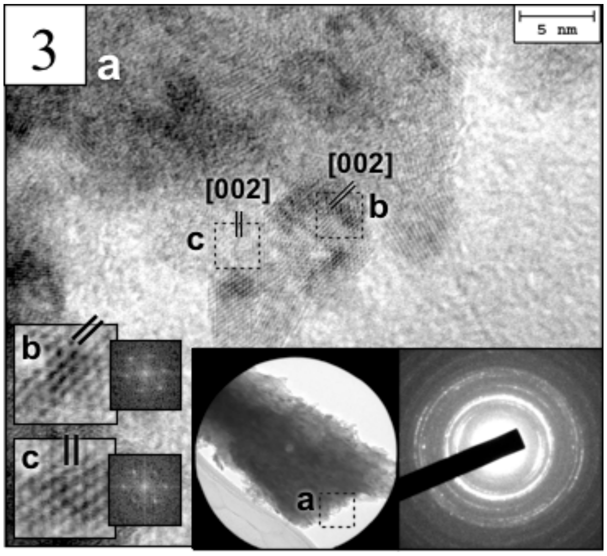

FIG. 3. Indexed lattice fringes correspond with bioapatite. Insets depict microfibril structure image with region of interest, **a**, diffraction pattern, and enlarged regions **b** and **c** with corresponding FFT diffractograms.

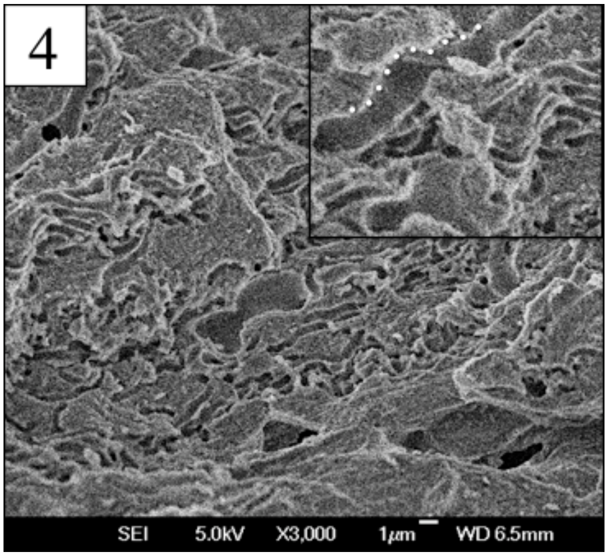

FIG. 4. SEM image of bacterial bioerosion in fossil bone. Insert of enlarged region depicts high contrast channel walls due to remineralization.

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