

## OBSERVATION OF KINETIC RESPONSE OF MINERAL AND COLLAGEN PHASES IN BONE BY X-RAY DIFFRACTION

A bone has a hierarchical structure as shown in Fig. 1 [1]. Macroscopic bone properties are dependent on the microstructure and properties of structural elements. The bone is a composite material that consists of hydroxyapatite particles in a matrix of collagen fiber. Borsato *et al.* [2] measured the strain of hydroxyapatite by X-ray diffraction and obtained clear evidence of the stress concentration on hydroxyapatite in the bone. Sasaki *et al.* [3] reported the stress-strain curve for tendon collagen by X-ray diffraction. However, the relationship between the mechanical behavior of hydroxyapatite and that of collagen has been unclarified. In this study, the microscopic mechanical behavior of bone as a composite material was examined by measuring the strains of hydroxyapatite and collagen by wide-angle X-ray diffraction and small-angle X-ray scattering, respectively.

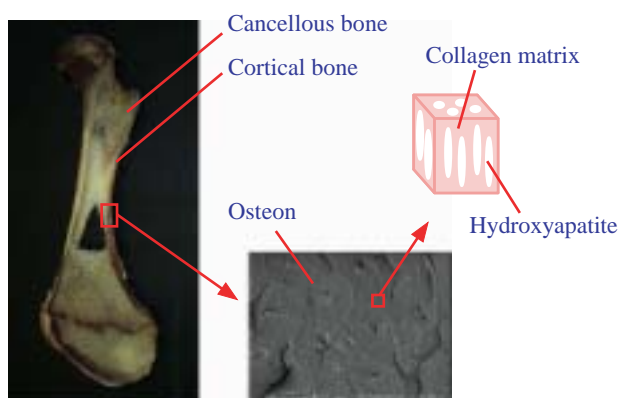


Fig. 1. Hierarchical structure of bone.

Twelve cortical bone specimens with an average size of  $40 \times 5 \times 0.5 \text{ mm}^3$  were cut out from the mid-diaphysis of the bovine femur using a diamond cutter. The longer edges of the specimen were parallel to the longitudinal axis of the femur. Before the experiments, the specimens were preserved in a saline solution.

X-ray energy was 15.0 keV at beamline BL40XU. The experimental setup is shown in Fig. 2. The X-ray path length was about 9 cm in wide-angle diffraction for hydroxyapatite (Fig. 2(a)), and 3 m in small-angle scattering for collagen (Fig. 2(b)). The X-ray diffraction pattern was recorded with an X-ray image intensifier (Hamamatsu Photonics, V5445P) and a fast CCD camera (Hamamatsu Photonics, C4880-80). The time resolution was 30 frames/sec. The specimens were subjected to a load using tensile loading apparatus. The tensile loading was synchronized with the X-ray shutter and CCD camera.

Figures 3(a) and 3(b) show the images of X-ray diffraction from hydroxyapatite and collagen in the bone, respectively. In the wide-angle diffraction from hydroxyapatite in the bone, diffractions from (211) and (002) are strong (Fig. 3 (a)). As the (002) plane of hydroxyapatite in the bone has a preferred orientation in the bone axial direction, most of the c-axis of hydroxyapatite crystals is parallel to the bone axial direction. In the small-angle scattering from collagen in the bone, diffraction from  $d = 22.6 \text{ nm}$  is strong (Fig. 3 (b)). The small-angle scattering pattern also contains a diffuse scattering perpendicular to the bone axial direction. Matsushima *et al.* [4] reported that this anisotropic diffuse scattering can be explained by the

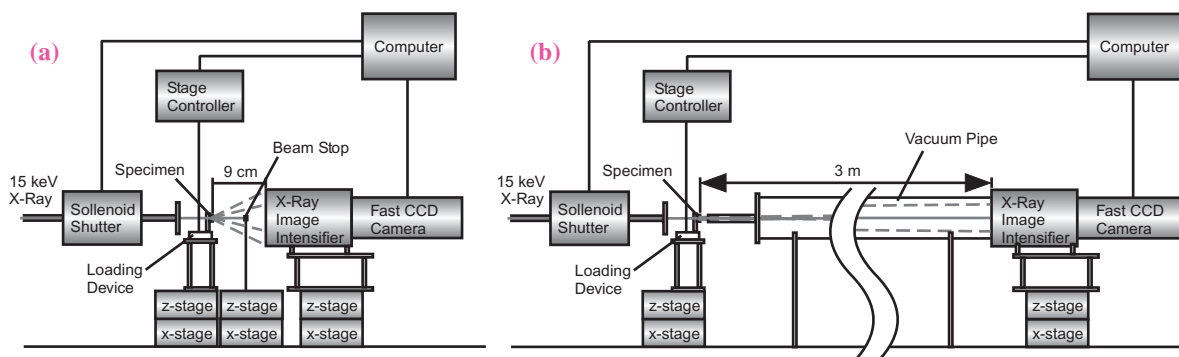


Fig. 2. Experimental setup for X-ray diffraction. (a) Wide-angle X-ray diffraction from hydroxyapatite in bone. (b) Small-angle X-ray scattering from collagen in bone.

shape and orientation of hydroxyapatite crystals.

Figure 4 shows the strain change of hydroxyapatite and collagen in the bone against the dynamic stress macroscopically applied to a bone specimen. The maximum strain of collagen is higher than that of hydroxyapatite. However, the difference in elastic modulus between hydroxyapatite and collagen is larger than that of the maximum strain. Therefore, it is estimated that hydroxyapatite is subjected to a larger stress. The strain of collagen changes quickly against the applied stress, however the strain of hydroxyapatite changes slowly. It is estimated that this difference in behavior is caused by the difference in elastic modulus between hydroxyapatite and collagen.

In summary, we could observe the mechanical behavior of hydroxyapatite and collagen in bone by wide-angle X-ray diffraction and small-angle X-ray scattering. A further study is now in progress to estimate the mechanical constitutive law for the hydroxyapatite – collagen composite material.

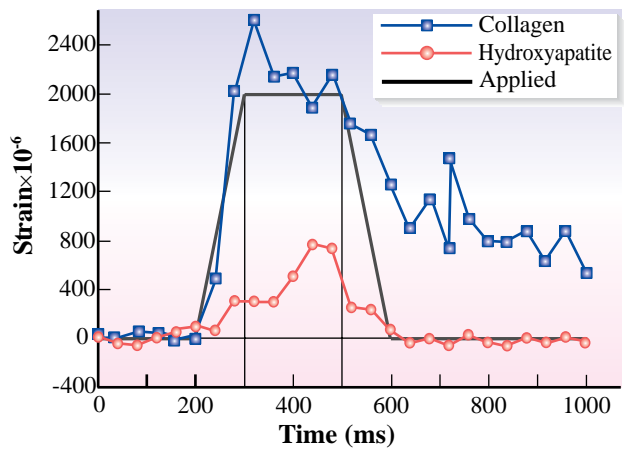
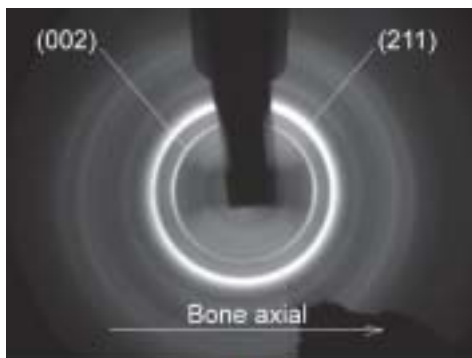
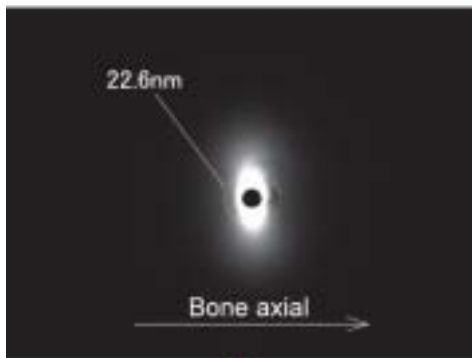


Fig. 4. Mechanical behavior of hydroxyapatite and collagen in bone by X-ray diffraction against macroscopically applied dynamic stress.



(a)



(b)

Fig. 3. X-ray diffraction images. (a) Wide-angle X-ray diffraction from hydroxyapatite in bone. (b) Small-angle X-ray scattering from collagen in bone.

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