ESRF	**Experiment title:** APATITE CRYSTALS AND COLLAGEN FIBRILS ORIENTATION IN OSTEONIC LAMELLAE	**Experiment number**: LS-1855
Beamline:	**Date of experiment**: from: 26-09-01 to: 29-09-01	**Date of report**: 18-06-01
Shifts:	**Local contact(s)**: Manfred BURGHAMMER	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

A. BIGI*

S. PANZAVOLTA*

University of Bologna, Italy

Report:

Single osteons, the structural units of compact bone, are cylindrical structures about 0.2 mm in diameter comprising concentric lamellae around a Haversian canal, and can be described as “longitudinal”, “transversal” or “alternate”as a function of the course of calcified collagen fibrils in successive lamellae. Structurally different osteons display different mechanical properties. This investigation addresses the long-standing question of whether the orientations of collagen bundles and hydroxyapatite crystals differ between the two types of human secondary lamellae, those that appear dark and those that appear bright under circularly polarizing light on alternate osteons’ transverse sections. A new technique, derived from an approach that Ascenzi A. et al. introduced in 1973 [1], is employed to isolate and prepare specimens of the two lamellar types. At variance with the previous method, the technique allows to obtain lamellar specimens free from residual portions of adjacent lamellae.

The study was performed using the scanning diffractometry setup of the ID13 microfocus beamline. The beam wavelength of 0.964 Å was obtained with a Si(111) monochromator and focused to 7 μm. 10 dark and 10 bright lamellar specimens, and 2 longitudinal osteonic hemisections were examined. The samples were glued to TEM grids, and the grids were mounted on a goniometric head, with the larger surface of the specimen perpendicular to the X-ray beam. Square areas of 25μm x 25μm or 20μm x 20 μm, as well as horizontal and vertical linear scans, were examined with spatial resolutions of 5 and 10 μm.

Figure 1a shows a typical SAXS pattern from an osteonic dark lamella, which displays a diffuse scattering shape elongated along the horizontal direction, and a discrete reflection corresponding to a periodicity of about 22 nm. The discrete reflection can be identified as the third order reflection of the axial periodicity of 67.0 nm of collagen, in agreement with previous results obtained on osteonic specimens [2,3]. The third order reflection of collagen appears preferentially oriented parallel to the specimen width, in agreement with a preferential orientation of collagen fibrils parallel to the osteon axis. The diffuse SAXS scattering is due to the inorganic crystals, and its shape is related to the distribution and orientation of the crystals inside the specimen: the diffuse scattering elongated along the specimen length (Fig. 1a) indicates that the crystals are preferentially oriented along the specimen width, that is parallel to the osteon axis. In agreement, the WAXS 002 reflection appears preferentially oriented along the specimen width (Fig. 1b), in agreement with a preferential orientation of the hydroxyapatite crystals with their c-axes aligned along the specimen width. The integrated intensity distribution along the Debye ring of the reflection is reported as a function of the azimuthal angle in Figure 1c.

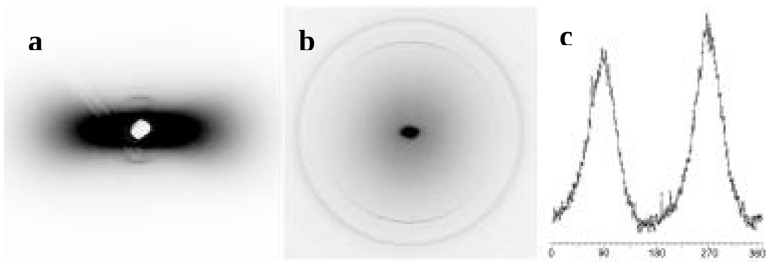
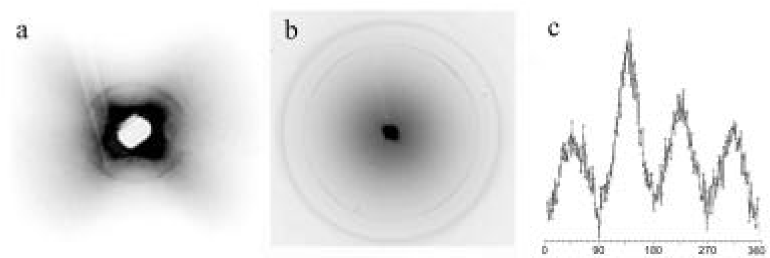


Figure 1

Bright lamellar specimens show dominant bidirectional orientation of both collagen and hydroxyapatite crystallites. Most of the patterns display a diffuse scattering elongated along two almost orthogonal directions. The two directions of blackening of the diffuse scattering are at about 45° with respect to the specimen width, suggesting that the hydroxyapatite crystals are preferentially oriented with their c-axes along two mutually orthogonal directions at 45° with respect to the lamellar width. In these patterns, the collagen third order reflection appears preferentially oriented along the same directions of blackening of the diffuse scattering (Fig. 2a). The 002 hydroxyapatite reflection in the WAXS pattern recorded from the same point (Fig. 2b) appears preferentially oriented along the same two directions, confirming that the c-axes of the hydroxyapatite crystals are preferentially oriented parallel to the collagen fibrils, and distributed along two mutually orthogonal directions at about 45° with respect to the specimen width. The two directions of orientation are evident in the integrated intensity distribution along the Debye ring of the reflection reported as a function of the azimuthal angle in Figure 2c.

Figure 2



The comparison of these data with those of the parallel investigation carried out by polarizing light microscopy and confocal microscopy leads us to suggest that alternate osteon consists of a rather continuous multidirectional structural pattern, where dark and bright lamellae display different mechanical and possibly biological functions.

References

[1] Ascenzi, A., Bonucci, E. and Simkin, A. (1973) *J. Biomech.* 6, 227-235.
[2] Ascenzi, A., Bigi, A., Koch, M.H.J., Ripamonti, A., and Roveri, N. (1985) *Calcif. Tissue Int.* 37, 659-664.
[3] Ascenzi, A., Benvenuti, A., Bigi, A., Foresti, E., Koch, M.H.J., Mango, F., Ripamonti, A., and Roveri, N. (1998) *Calcif. Tissue Int*. 62, 266-273.