ESRF	**Experiment title: Microfocus X-ray diffraction of a single collagen fibril (block allocation of 3 projects)**	**Experiment number**: LS-1271
Beamline: **ID22**	**Date of experiment**: from: 28.4.99 to: 3.5.99	**Date of report**: 14Feb 00
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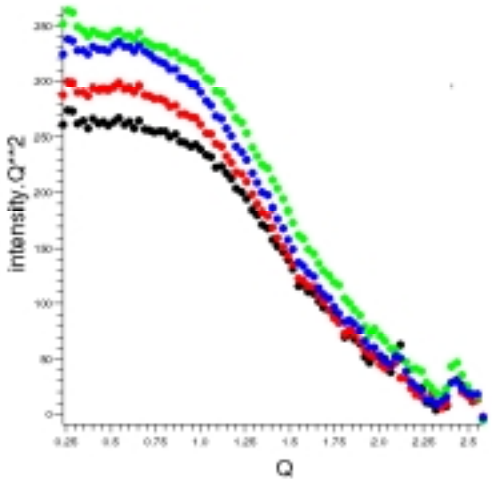
Report:. Here we outline three current projects that we are investigating using the microfocus beamline at ID22 of the ESRF in beamtime allocation LS1271.

Calcified tissue: The basis for mineralisation in bone tissue remains unresolved -at a molecular level. The formation of plate like or needle crystals is thought to derive from interaction with the collagen matrix. Specific interactions may lead to the formation of nm scale structures at the nascent edge of growing bone. Previous investigation by our group have shown that the detectable edge of bone development occurrs over a very small scale at the edge of bone growth possibly as little as 5-10 microns. The use of small angle X-ray microfocus technology has allowed us to obtain scattering data relating to the habit of the crystallites at the nascent edge of growing and senescent bone. The technique therefore allows the investigation of bone growth mechanisms in situ without any extraction procedure that may produce artefacts. Experiments at ID22 showed that the high brilliance of the beamline allowed 2-5 micron thick structres to be diffracted in 2-5 minutes. The results indicated that the earliest detectable structues are plate like and far thinner than mature tissues. The data has formed part of the ESRF highlights for 2000.

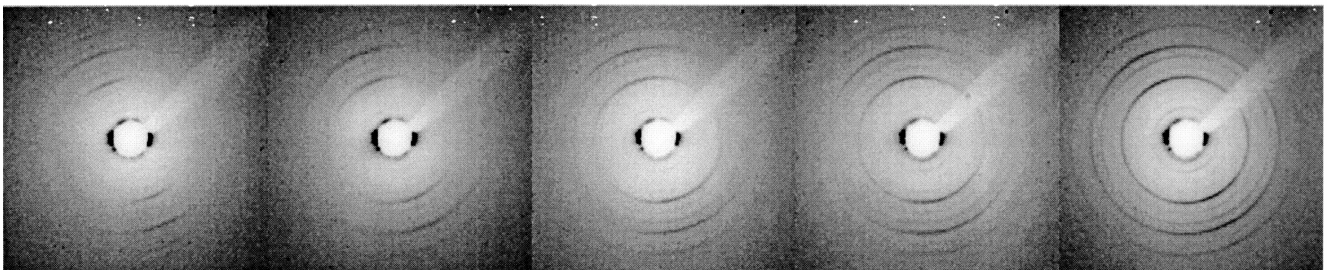


A Kratky plot $I*Q^2$ vs Q of the scattering profile from a 16 week old turkey leg tendon sample taken at 5 micron intervals from the detectable edge of mineralisation, the scattering profile changes indicate an increase in the mineral particle size from 1.7-2.25nm. The shape of the profile at low angles indicates the shape of the crystals to be plates.
Data shown from bottom to top 5 micron after edge; 15micron after edge; 15 micron " ; 25micron "

Collagen: The molecular packing within a collagen fibril remains to be fully determined [2] [3]. The structure function relationship of fibrillar collagen has so far relied on assumptions about the interactions between fibrils within a tissue, such interactions have inherent visco elastic properties of their own. It was the aim of our research group to develop technology to obtain diffraction images from a single fibril of collagen and then to extend the study to measuring the biomechanical properties in conjunction with diffraction data. Although we were unable to diffract a single collagen fibril, we were able to obtain diffractino from 1 micron thick sections of tendon indicating the feasibility of the project. The accuracy of positioning and immobilisation of the sample require to be improved. Collagen fibrils typically have a diameter of less than 1 micron. They therefore present a benchmark in the future success of X-ray microfocus technology.

Toughening mechanisms in skin: Connective tissues such as skin and artery are collagen fibre-reinforced composites that can accommodate large deformations. It is of considerable interest to understand why tissues such as skin are so good at avoiding fracture. This may provide answers as to the basis of genetic defect/medical conditions which result in connective tissue damage, leading to dysfunction and sometimes death and to learn

mechanisms for toughening from nature that may be engineered into advanced composites [4]. In skin it is believed that fibril reorientation occurs at the edge of a fracture, or wound. The process occurs over a very short range (approx. 100microns) and therefore microfocus is required to make studies of the collagen orientation in the fully hydrated state. The reorientation effects were observed and the data is being prepared for publication.



X-ray diffraction of the meridional pattern of skin showing changes an increase toward isotropy as the beam was moved away from the edge of an incision.

References:

[1] P. Fratzl, S. Schreiber and K. Klaushofer, *Conn. Tissue Res.* 34, 247-254 (1996).

[2] Wess T.J., Hammersley A.P., Wess L., Miller A. *J.Mol. Biol.*, 275, 255-267 (1998).

[3] Orgel J.P., Wess T.J.,Miller A. *Structure* 8, 137-142 (2000).

[4] In Composite Materials : Engineering and Science Matthew F.L. and Rawlings R.D. 327-331 Publishers Chapman and Hall. (1994).