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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** The Micromechanical Properties of Articular Cartilage: Their Structural Bases and Role in Osteoarthritis	**Experiment number**: SC-1466
Beamline: ID18F	**Date of experiment**: from: 02 April 2004 to: 06 April 2004	**Date of report**: 18 February 2005
Shifts:	**Local contact(s)**: Dr. Raymond BARRETT	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Introduction:

In synovial joints the bone is covered by a thin layer of articular cartilage that provides a low-friction bearing surface and acts as a shock-absorber. The main structural element of cartilage is a highly organised network of Type II collagen fibres, maintained in tension by an osmotic, or swelling, pressure generated by a viscoelastic gel of highly anionic proteoglycans that fills the voids in the collagen network. The collagen fibres run parallel to the articular surface in the superficial layer of cartilage, are isotropically oriented in the mid zone and, in the deep zone overlying the bone, run perpendicular to the interface (Stockwell 1979).

The interface between cartilage and bone comprises a zone of calcified cartilage bounded above by the tide mark and below by the cement line. The micro-structural organisation of the zone of calcified cartilage is believed to be similar to that of cartilage with Type II collagen fibrils arranged predominantly perpendicular to the articular surface except that it is mineralized. The mineral comprises mainly of calcium apatite, but its organisation and relationship to collagen fibres have only recently been reported (Zizak et al 2003). This layer is believed to be a mechanically important interface in matching the disparate properties of bone and cartilage. Hence, or otherwise, it may be a key element in the development of osteoarthritis and osteoporosis and, indeed, may explain the inverse correlation in the incidence of these conditions.

The aims of our research were first to explore the potential of microbeam SAXS and WAXS to further chacterise the structure of the interface in diseased tissue and second to establish whether these techniques can be used to quantify structural changes associated with the application of mechanical loads.

Methods:

A cross section of bone and articular cartilage, cut longitudinally and approximately 1mm thick, was taken from the metacarpalphalangal joint of the horse. This was done immediately post

mortem from a horse undergoing euthanasia. WAXS and SAXS diffraction patterns were obtained at 15 equally spaced depth increments of 67μm passing from the cartilage surface through the calcified cartilage into the bone to a maximum depth of 1mm both in normal tissue and through a lesion. Loads were then applied to the cartilage surface using a custom built press and diffraction patterns were again obtained at increasing depths through the sample. Additional samples that had been decalcified were also studied. This was to allow clear identification of the d-spacings assigned to collagen and crystal components from which the bone and cartilage consists.

Results:

The experiments provided a rich body of data and a PhD student has been engaged in the study who is now completeing the analysis. WAXS diffraction patterns have been analysed using fit2D and, by observing the d-spacings found in cartilage and bone of the decalcified sample comparing to the calcified sample, attribution was made of the d-spacings associated with the collagen and crystal as shown in table 1 below. The three main regions, bone, interface and cartilage could then be identified by looking at the d-spacings found in the scan.

Crystal d-spacings (Å)	Collagen d-spacings (Å)
1.8	2.8
3.4	3.1
3.8	3.2
5.2	2.3
8.2	6.2

Table 1: D-spacings associated with collagen and mineral (calium apatite).

Firstly, an analysis was made of how the orientation of the components changes in bone, calacifed cartilage and cartilage. Figure 1 illlustrates a significant change in orentation of the [100] plane of calcium apatite from bone to the interface region and figure 2 illustrates a change in orientation of collagen (d=6.2Å) from calcified cartilage to cartilage.

When the sample was loaded significant changes were observed on the WAX and SAX diffraction patterns with regard to orientation, intensity and width of bands at all depths in the specimen. The intensity of the rings on the diffraction patterns, particularly d=3.2Å and d=2.8Å for the interface region, decreased with load and they became more highly oriented as shown in figure 3. The quantitive analysis of these results is currently in hand but a preliminary anaylsis indicates changes in orientation with load propagate deep into the tissue.

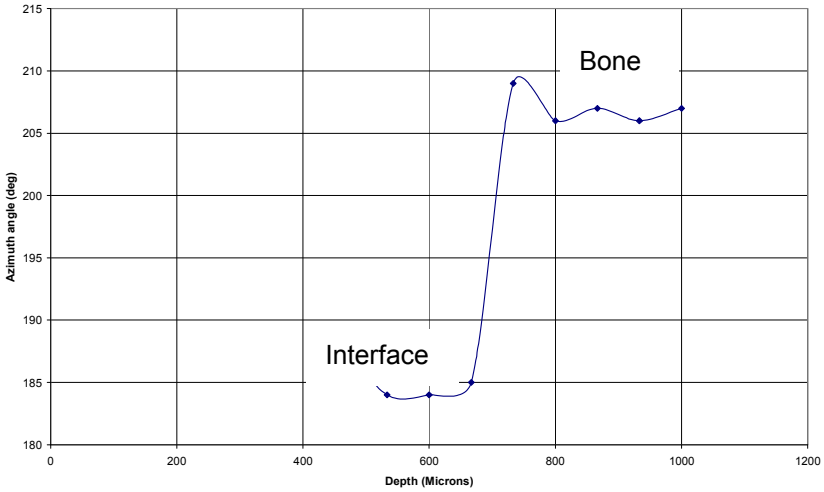

Figure 1: The change in orentation of the [100] plane of calcium apatite with depth into the sample

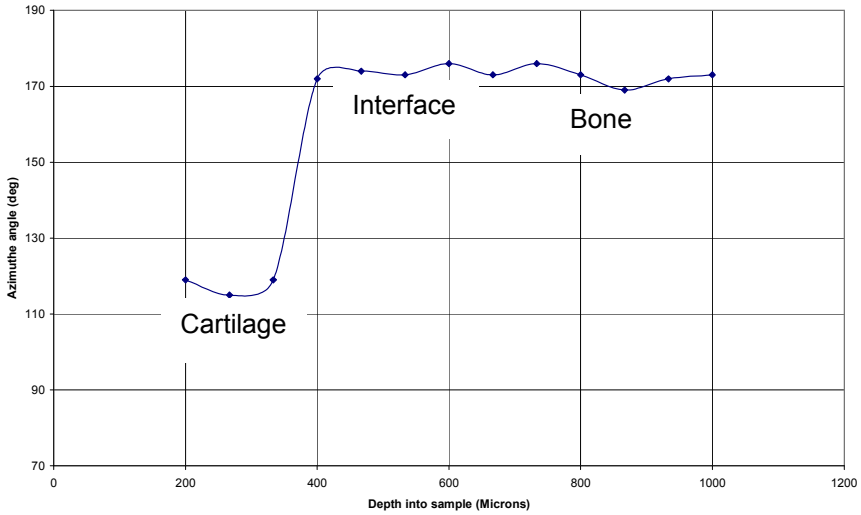

Figure 2: The change in orentation of the collagen (d=6.2Å) with depth into the sample

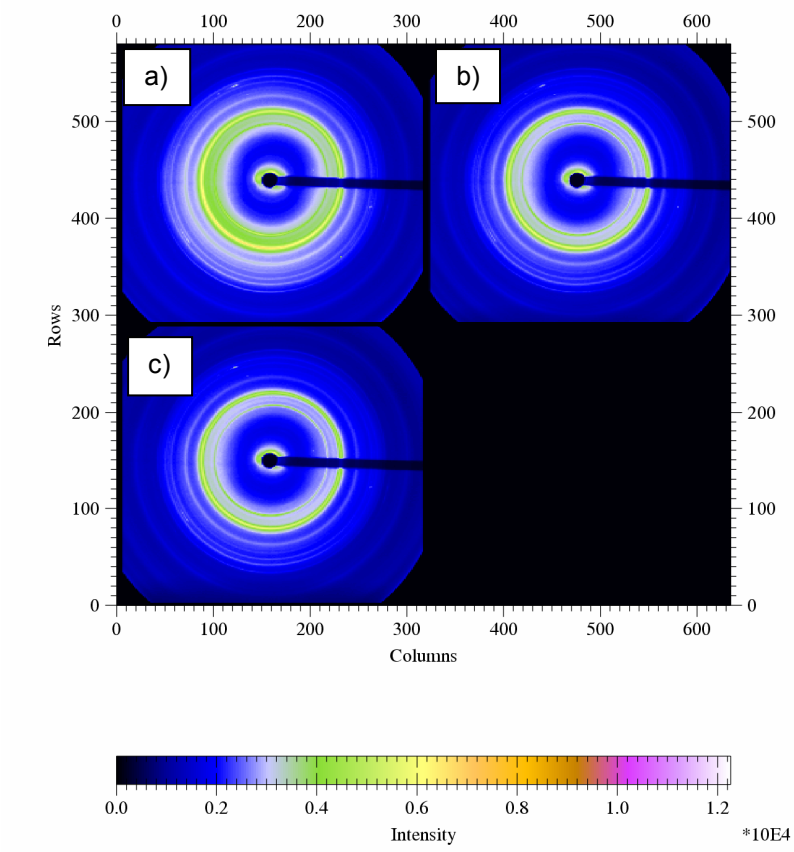

Figure 3: WAXS diffraction patterns of the interface region when a) unloaded, b) and c) with load in the physiological range.

Conclusions:

Although the data analysis from this rich data set is still to be completed, it is clear that new insights into the structure of both the normal and diseased interface region are emerging. In addition, we have detected changes with load, propagating deep into the tissue. Characterisation of the time-course of these changes would greatly advance understanding of tissue mechanics and warrants further investigation.

References:

Stockwell RA. 1979. *Biology of Cartilage Cells*: C.U.P. Cambridge

Zizak I, Roschger P, Paris O, Misof BM, Berzlanovich A, et al. 2003. Characteristics of mineral particles in the human bone/cartilage interface. *J of Structural Biology* 141: 208-17