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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 Cartilage and the Bone-Cartilage Interface: Changes with Age and Disease	**Experiment number**: MD-189
Beamline: ID18F	**Date of experiment**: from: 1st December 2005 to: 5th December 2005	**Date of report**: 25th May 2006
Shifts:	**Local contact(s)**: Dr. Pierre BLEUET	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

D. A. Bradley, K.M.Knapp*, C.J.Moger*, C.P. Winlove*
School of Physics, University of Exeter

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 tangential layer of cartilage, are isotropically oriented in the transitional zone and, in the radial 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. In the calcified cartilage mineralised Type II collagen fibrils are arranged predominantly perpendicular to the articular. 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.

The aims of our research were to further chacterise, using SAXS, the structure of articular cartilage and the calicifed cartilage in different regions of the equine metacopophalangeal joint and explore the relationships between the structural differences regional differences in joint loading. A second aim was to quantify structural changes associated with the application of mechanical loads to the joint. The final aim was to characterise cartilage structure in osteoarthritic lesions and to compare the loading response of lesions to that of healthy tissue.

Methods:
A cross section of bone and articular cartilage, cut longitudinally and approximately 1-2mm thick, was taken from the metacarpolphalangal joints of horses, immediately post mortem in horses undergoing euthanasia. The samples were mounted in a custom-made loading apparatus on beamline ID18F and SAXS diffraction patterns were acquired at 25μm interverals passing from the

cartilage surface through the calcified cartilage into the bone. Measurements were made at different sites around the articular surface including both normal tissue and lesions. At each location a series of measurements were performed as increasing compressive loads were applied to the cartilage surface. A small number of area scans were performed in order to investigate the lateral spread of strain through the joint under the applied load. Area scans were also performed on lesions to investigate local changes in micromechanical properties.

Results:
The experiments provided a rich body of data. The analysis of collagen structure has been completed and the analysis of the mineral component is in progress.

Regional variations in collagen structure have been revealed over the articular surface, which can be correlated with the regional loading patterns in-vivo. Notably, areas of the joint that receive the most load appear to have greater order in the radial zone. In lesions this order is lost with the progresion of the lesion. Changes also occur in cartilage structure as deep as the subchondral bone and these changes are especially apparent in samples where the cartilage surface has been lost due to severe progression of disease. These findings have been accepted for presentation at the World Congess of Biomechanics and full papers will be submitted shortly.

The effects of load on collagen organisation have also been analysed. In normal tissue, the transitional and tangential zones of the cartilage compress initally when a surface stress of approximately 60 kPa is applied. This compression reaches a limit above this load and with further increase in load the fibres in the deep radial zone, as deep as the zone of calified cartilage, reorientate and there is a loss of order, tentatively ascribed to fibre ‘buckling’.

Applying comparable loads to lesions caused fibre breakage in the upper radial zone and fibre buckling was not observed implying that fibre breakage prevents the propagation of strains into the deeper tissue. These findings are also being prepared for publication.

Conclusions:
Although the data analysis is still incomplete and important questions relating to the mechanical coupling of collagen and mineral remain to be addressed the preliminary analysis has provided important new insights into cartilage micromechanics that would be difficult to obtain by other techniques.

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

Publications:

Moger C J, Barrett R, Bleuet P, Ellis R E, Knapp K M and Winlove C P, 2006. Mechanical Properties of Cartilage under load determined using Small angle X-ray diffraction (SAXS) and Raman micro-spectrometry, 2006, 5th World Congress in Biomechanics, Munich

Moger C J, Barrett R, Bleuet P, Bradley D A, Ellis R E, Green E M, Knapp K M, Muthuvelu P, Winlove C P, 2006. Regional Patterns of Collagen Orientation in Normal Articular Cartilage and Subchondral Bone and in Lesions of the Equine Metacorpophalangeal Joint Determined using X-ray Diffraction. To be submitted to Osteoarthritis and Cartilage

Moger C J, Barrett R, Bleuet P, Ellis R E, Knapp K M and Winlove C P, 2006. Collagen re-orientation in Cartilage using Small angle X-ray diffraction and Polarized light. To be submitted to Journal of Biomechanics