ESRF	**Experiment title: Structural studies of extracellular supramolecular assemblies**	**Experiment number**: SC963
Beamline: 1D02/1D18F /ID13	**Date of experiment**: from: Apr 02 to: Jun 03 Interim report	**Date of report**: 25.8.03
Shifts: LTP	**Local contact(s)**: Drakopoulos/Roessle	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Please note changes in personnel and location have altered this list.

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Report:

This is an interim report for the research carried out on an LTP where the research group was originally based at Stirling. The group has now relocated to Cardiff as of 1.9.03.

The proposal was devised as 4 subprojects progress in each is dealt with here.
The LTP has already resulted in publications in press and there are also a number of manuscripts submitted for publication.
Subproject 1 Collagen structure function relationships in a single fibril. The main aim using microfocus beamlines was to obtain X-ray diffraction images from a single collagen fibril, during the last year, we have developed techniques for fibril dispersement and then isolation in standard protein crystallography cryo-loops. Fluorescent labelling of fibrils has allowed the position of fibrils to be mapped within a loop and we believe that we have now obtained weak diffraction images from single fibrils. These are not of sufficient quality to discriminate between packing models and require improvement. This work has been included in a review of the structure of collagen and is in press. **Laing J. Cameron G.J. Wess T.J. Molecular structure, dimensions and axial order of the type I collagen fibre in press Research Signposts July 2003.** Work will continue to build on this to improve signal to noise and to obtain better images from whole tendon samples. Cryocooling of fibril bundles has been used to distinguish the contribution of thermal and static disorder in fibril fibre diagrams. There is a significant alteration in the distribution of diffuse scatter in cryo-cooled samples whilst the Bragg reflections are maintained**. This work in preparation for submission to Journal of Molecular Biology.**

Subproject 2 Structure function relationships of fibrillin and a basis for molecular elasticity. Beamline ID02 has yielded excellent results that have allowed us to determine the alterations in the supramolecular packing of fibrillin microfibrils. In conjunction with studies of Raman spectra of extended and relaxed fibrillin rich tissues, we have been able to show that the elastic response in extensions of up to 150 % rest length reveal alterations at of molecular hierarchies from secondary structure to interfibrillar levels. **This work is now in press Haston J.L. , Engelsen S.B. Roessle M. , Clarkson J, Blanch E.W. , Baldock C, Kielty C.M. , Wess T.J. Raman microscopy and X-ray diffraction: A combined study of fibrillin-rich microfibrillar elasticity in press Journal of Biological Chemistry vol 278 August 2003.** The research into the early stages of deformation of fibrillin rich tissues on ID02 has also shown that the relative orientation of fibrillar bundles is a significant contributor to the initial non-linear elastic response. This work has been included in a book chapter **Kielty CM Wess TJ Haston JL Sherratt M Shuttleworth CA Organisation and biomechanical properties of fibrillin rich Microfibrils (2003) in Marfan Syndrome Edited by Robinson PN and Godfrey M. Pub. Landes Bioscience Georgetown Texas.**
We have also made an investigation in to the fibrillin rich microfibril samples of aorta from a variety of invertebrates. This has produced some initial results indicating scattering from fibrils of a similar diameter to vertebrate microfibrils, however little axial ordering is observed.

Subproject 3 Methods of diagenesis and nucleation in calcifying tissues
Two-dimensional microfocal mapping of a variety of archaeological bone samples has allowed us to determine the size and shape of nanocrystallites within archaeologically important samples. Our work (using bulk samples) has shown that the SAXS signal from bone nanocryatallites is the best indicator of bone nano-preservation and can be used to screen bone samples for the success of endogenous DNA extraction. Using microfocus studies of archaeological cave bone and contemporary bone, we have shown that microregions of intact bone can be surrounded by more degraded material. This brings the possibility of in situ micro DNA extraction a step closer. The work on two-dimensional scans from a number of diary cattle bones is in preparation for submission to Journal of Archaeological Science. We have also examined a number of mummified bones from Cladh Hallan Burials of South Uist Scotland; these have attracted media interest since they are the first indication of mummification in European culture. Microfocus studies reveal that there is an ingress of mineral modification that halts abruptly is novel and presumably due to the mummification process of short-term peat bog burial this work is being submitted to a general interest science journal as part of the larger study. Our automated data analysis techniques are now being used by the research group of Winlove from Exter (UK) for data collected at ESRF. We have yet to attempt tomography studies.

Subproject 4 Mechanisms of deterioration in historic parchment
Microdiffraction studies at ID18F have been used to map the changes in the fibre diagram of collagen from historic parchment. This has allowed the local effect of 1) writing media such as iron based inks 2) laser cleaning of historical documents on the deterioration of collagen to be assessed. Methods have already been established to determine the collagen: gelatin ratio in a fibre diagram, essentially as crystalline index approach which we are currently using. We are developing a more rigorous analysis based on Principal Component Analysis to determine the base functions that comprise each fibre diagram. Samples of historic parchment were obtained from the National Archive for Scotland and from IDAP the EU 5th framework project for the damage assessment of parchment.
Iron based inks are known to have a macroscopic degradative effect on the integrity of historic parchment. This effect has now been studied by microfocus X-ray diffraction in sections underneath lettering and adjacent to lettering in the plane of the page. Laser cleaning techniques are gaining popularity in conservation practice since they are chemical agent free, non-contact methods. It is an effective way of removing dirt from the parchment surface. The long-term effect of laser cleaning depends on its effect on the collagen structure underneath the surface dirt. In conjunction with the Museum Conservation Centre in Liverpool, we have produced a number of trial samples from which we have made thin sections. X-ray diffraction indicates that low wavelength (UV) laser cleaning does have an effect on the structure of surface collagen, however longer wavelengths that are still effective in the cleaning process do not produce a damaging effect as judged by our X-ray diffraction studies. This information has been used to guide the Conservation centre in their procedures for cleaning. A manuscript on the laser cleaning technique has been

submitted to Applied Surface Science (Aug 2003). A manuscript on to the effect of ink on parchment integrity is in preparation.