EUROPEAN **S**YNCHROTRON **R**ADIATION **F**ACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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:** Relating ultrastructure to curvature in normal and pathological corneas	**Experiment number**: MD-434
Beamline: ID13	**Date of experiment**: from: 30 /09/2009 to: 02/10/2009	**Date of report**:
Shifts: 9	**Local contact(s)**: Dr. Manfred Burghammer	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Prof. Keith M. Meek (Main applicant), Structural Biophysics Research Group, School of Optometry and Vision Sciences, Cardiff University, Cardiff, UK

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Report:

Aims and background information:

- To compare different forms of cross-linking (i.e. UVA/Riboflavin, glycaraldehyde, glutaraldehyde, carbodiimide (EDC) and transglutaminase) to see the effect of the procedure on the intermolecular spacing of collagen at different levels of hydration.

- To complete the preliminary work we carried out in the past at the ESRF, to show how collagen lamellae from the central cornea integrate with those in the periphery of the tissue, and understand further the role of collagen in the maintenance of normal corneal curvature.

Methodology

Correlation of different corneal cross-linking methods in relation to hydration

Ovine corneal strips were crosslinked using five different agents (i.e. UVA/Riboflavin, glycaraldehyde, glutaraldehyde, carbodiimide (EDC) and transglutaminase). A 4x4 mm central piece was dissected, weighed and exposed to the X-ray beam. Samples were then left to air dry for 1 and 2 hours and the weight/X-ray procedure was repeated. Finally, samples were dried for 3 days and the approximate hydration of samples was calculated and correlated to the changes in collagen intermolecular spacing.

Lamellar integration in the peripheral human cornea

A pair of normal human eye-bank corneas provided by the National Disease Research Interchange (NDRI), PA were examined. In our previous trip to the ESRF (SC-2246) we examined all eight meridians of a left human cornea, gaining information on how the number and direction of fibrils populating the two (centrally) orthogonal preferred directions changes as one moves from cornea to limbus. In our trip in September 2009 to the ESRF (MD-434) we collected excellent data following the same procedure from a further pair of normal human corneas.

Results

Correlation of different corneal cross-linking methods in relation to hydration

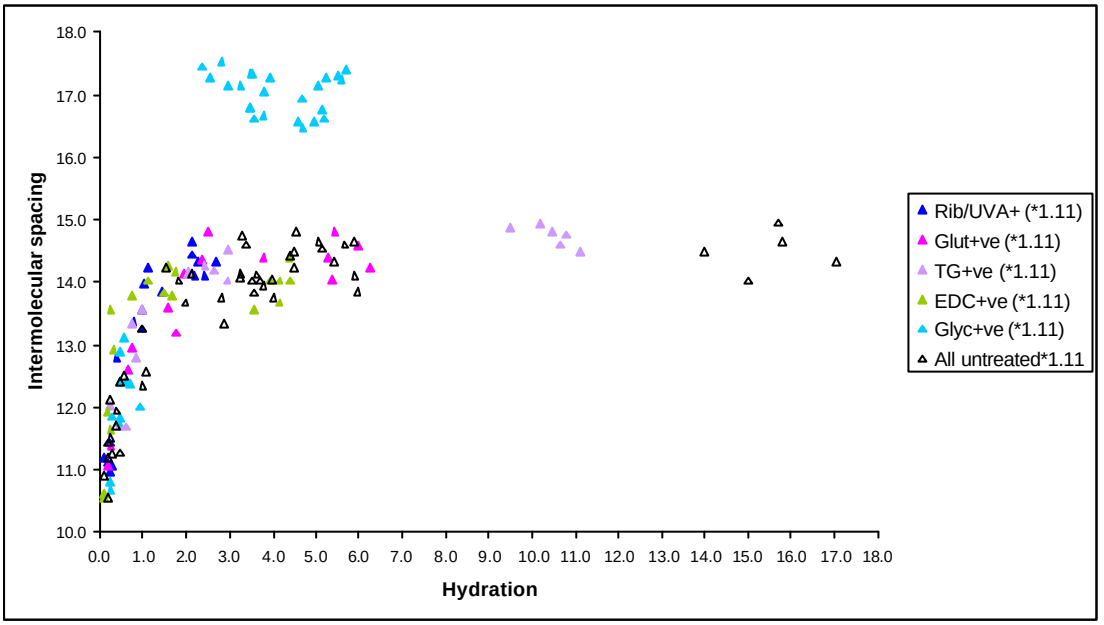

Figure 1: The intermolecular spacing variation after different types of cross-linking

Various methods of cross-linking have different effects on the comeal stroma. Our results demonstrate very clearly that this effect is dependant on the level of hydration in the tissue. For example, at low hydration levels (i.e. H<1) no collagen intermolecular spacing differences were observed between the different cross-linking treatments. Between hydration values of 1 and 6, glyceraldehyde had the most profound effect in increasing the intermolecular spacing of collagen within the corneal stroma ($p<0.01$). However, the fact that glyceraldehyde does not induce any profound differences on collagen intermolecular spacing at low hydration (H<1), below the critical level, shows that even this treatment of cross-linking does not prevent collagen collapse in the corneal stroma when the conditions become extreme.

Lamellar integration in the peripheral human cornea

The wide-angle set-up of ID-13 used during the current trip yielded excellent results for the lamellar integration studies. The additional samples that we scanned gave us the oportunity to complete the existing set of data that we obtained in our previous trip (SC-2246). The data are currently being analysed and this should be completed soon.