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Experiment Report Form

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Reports can 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.

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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:** Investigation of the short and long range organisation in photo-activated collagen hydrogels	**Experiment number**: MA-2796
Beamline: ID02	**Date of experiment**: from: 08.09.2015 to: 11.09.2015	**Date of report**:
Shifts: 6	**Local contact(s)**: Peter Boesecke	*Received at ESRF:*

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Introduction

The aim of this experiment was to investigate the structural organisation of a novel class of collagen hydrogels, interrogating both triple helix (d ~ 1 nm), and fibrillar (d ~ 100 nm) organisation of functionalised and photo-crosslinked collagen. These collagen hydrogels have already been reported to display tunable compression and swelling properties in physiological conditions,[1] whilst their wound healing capability has been proven in a recent *in vivo* study in diabetic mice. Preliminary Wide Angle X-ray Scattering (WAXS) suggested the preservation of collagen triple helices in the cosslinked networks, whilst no long-range features could be resolved and only dry-state (hardly describing the material organisation in biological environment) measurements were carried out. Addressing the collagen organisation in resulting hydrogels was therefore considered an important scientific and technogical question aiming to establish defined structure-property relationships and extend the applicability of this systems to other clinical (e.g. dental) settings.

Experiments

Dry collagen networks and corresponsing hydrogels were prepared via functionalisation with either 4-vinylbenzyl chloride (4VBC) or methacrylic anhydride (MA); a second set of samples constisted of wet-spun crosslinked collagen fibres.[2] Three types of measurements were carried out: (1) Small Angle X-ray Scatterring (SAXS) at varied sample-to-detector distances (0.7–5 m)[3] in order to resolve any triple helical or fibrillar collagen features in dry crosslinked materials. (2) SAXS on collagen materials with different hydration levels (via incubation in 0-50 wt.-% H_2O-EtOH solutions) in order to elucidate how the presence of

[1] G. Tronci, C.A. Grant, N.H. Thomson, S.J. Russell, D.J. Wood. *J. R. Soc. Interface* 2015, **12**, 20141079.
[2] M.T. Arafat, G. Tronci, J. Yin, D.J. Wood, S.J. Russell, *Polymer* 2015, **77**, 102-112.
[3] C.A. Maxwell, T.J. Wess, C.J. Kennedy, *Biomacromolecules* 2006, **7**, 2321-2326.

water affected the collagen organisation in the hydrogel state. (3) Real time SAXS on photo-active collagen solutions (0.8 wt.-% collagen) during UV irradition (0–60 min), aiming to monitor the material organisation during photo-induced network formation.

Results

SAXS measurements at short (0.7 m) sample-to-detector distance revealed a triple helix collagen conformation in dry crosslinked networks, bein these ones prepared as either film (Figure 1, left, black curve) or wet-spun fibre. A triple helix peak was clearly observed at q = 5.25 nm^{-1}, regardless of the network architecture introduced at the molecular level and material format, whilst no long range features was detected at long (2-5 m) sample-detector distance (Figure 1, left, grey line). These findings confirm the fact that the covalent functionalisation does not affect the triple helical structure of collagen; they also suggest that fuctionalised triple helices do not refold into fibrils in the crosslinked state. Possible reasons for this latter point can be: (1) presence of covalent junctions among collagen molecules, resulting in suppressed self-assembly capability;[4] (2) environmental conditions hindering collagen assembly;[5] (3) low concentration of functionalised collagen in the photo-active solution (0.8 wt.-%), which is way below the reported 150 $mg \cdot ml^{-1}$ concentration threshold at which fibrillar aggregation of collagen occurs.[6] Given that these collagen networks were prepared via direct UV irradiation of functionalised collagen solutions (or one-step crosslinking reation as in the case of wet-spun fibres) without a pre-conditioning at a fibrillogenesis-inducing pH, it may be anticipated that the absence of long-range collagen features in SAXS spectra could be attributed to a non-optimal sample conditioning rather than to the collagen functionalisation per se. This hypothesis has been supported by preliminary SEM investigations on collagen gels formed following incubation of functionalised collagen solutions (2 $mg \cdot ml^{-1}$) in phosphate buffered saline (PBS) solution.

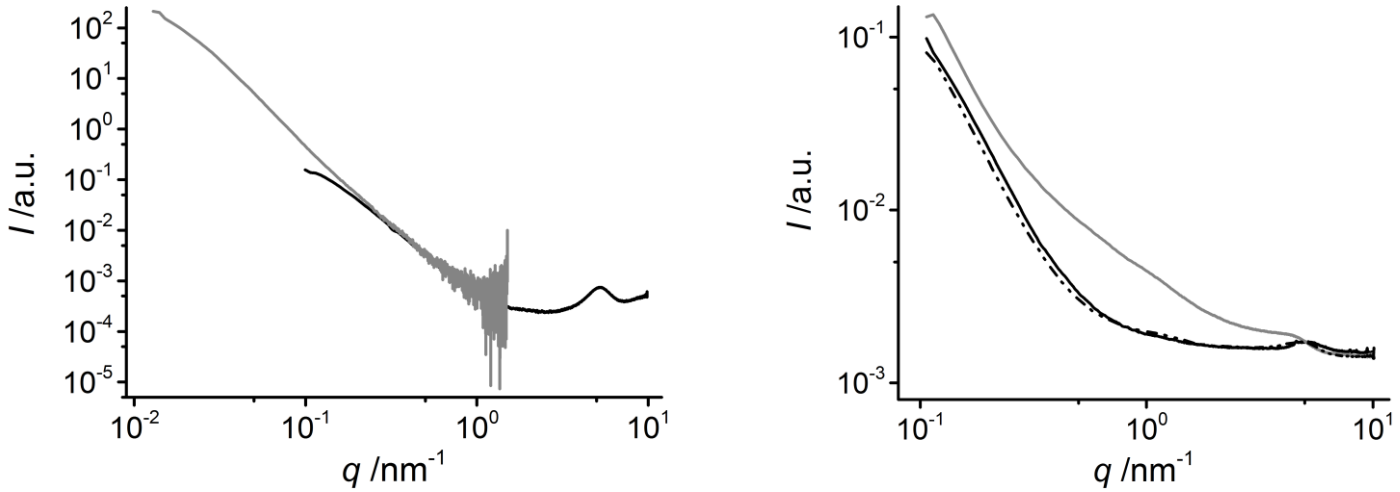

Figure 1. (Left): exemplary SAXS spectrum of a dry collagen network (CRT-4VBC25*) at either 0.7 (black curve) or 2 (grey curve) m sample-detector distance. Right: exemplary SAXS spectra of sample CRT-4VBC25* acquired with a 0.7 m sample-to-detector distance in the dry state (—), following intermediate incubation (15 min) in 1 vol.-% distilled water in ethanol (··–), and after dehydration (15 min) in 100 vol.-% ethanol (—).

Besides measurements in the dry state, SAXS spectra of water-swollen hydrogels displayed the absence of any triple helical or fibrillar peak. In order to elucidate this point, SAXS was carried out on dry samples before and after 15-min incubation in an ethanol solution containing 1 vol.-% distilled water. As observed in Figure 1 (right), spectra revealed a remarkable shift of the original triple helix peak position towards smaller *q* values following sample incubation in 1 vol.-% water-ethanol solution, whist a drastic peak broadening was

[4] G. Tronci, A.T. Neffe, B.F. Pierce, A. Lendlein, *J. Mater. Chem* 2010, **20**, 8875-8884
[5] J.M. Caves, V.A. Kumar, J.W.W. Cui, A. Martinez, R. Apkarian, J.E. Coats, K. Berland, E.L. Chaikof, *J. Biomed. Mater. Res. Part B Appl. Biomater.* 2010, **93B**, 24-38.
[6] F. Gobeaux, G. Mosser, A. Anglo, P. Panine, P. Davidson, M.-M. Giraud-Guille, E. Belamie, *J. Mol. Biol.* 2008, **376**, 1509-1522.

observed (grey line) in these conditions. Furthermore, when the same sample was dehydrated in ethanol, the triple helix peak was found to shift back to the original position as in the case of the native dry material. A similar situation has been reported in the case of PEG-diacrylate hydrogels, whereby the correlation peak position shifted to smaller *q* values following complete hydration. This was found to correlate with the increase in the distance between cross-link junctions in light of the water-induced swelling of the covalent network.[7] Also in light of other reports investigating collagen organisation at varied collagen concentration,[8] obtained SAXS data suggest that the disappearance of the triple helix peak following complete equilibration with water is unlikely related to a solvent-related scattering effect.

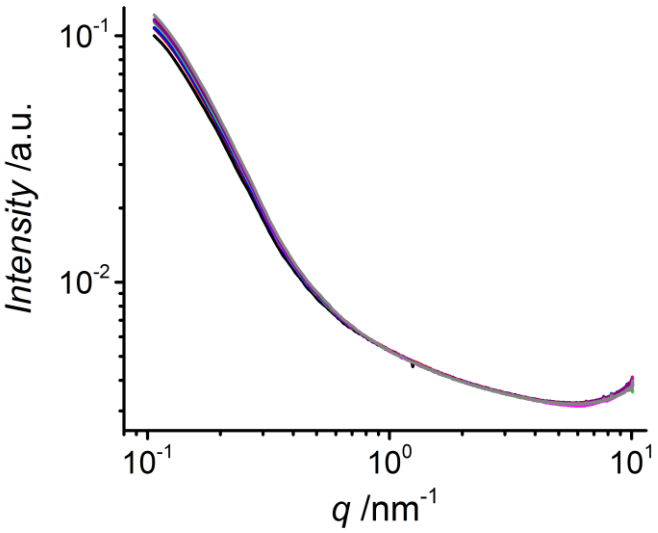

Figure 2. Exemplary SAXS spectra obtained during UV (365 nm) irradiation of a photo-active collagen solution (CRT-4VBC25) at 0 (black), 5 (red), 10 (blue), 15 (green), 20 (magenta), 30 (purple) and 40 (grey) minutes of UV irradiation.

Other than measurements on dry networks and hydrogels, real-time SAXS measurments on photo-active collagen solutions during UV irradiation were also carried out in order to investigate any structural variation during the UV-induced network formation. As observed in Figure 2, no peaks related to collagen features could be identified in resulting spectra, which in line with the results obtained with the water-swollen collagen networks (Figure 1, left). Interestingly, the intensity of the curve at q= 0.01 nm^{-1} was found to be progressively increased over the time of irradiation, suggesting the formation of covalent crosslinks between functionalised collagen triple helices following application of UV light and radical formation. This was macroscopically in agreeement with the conversion of the photo-active collagen solution into a water-stable hydrogel at the end of the real-time measurement.

[7] D.J. Waters, K. Engberg, R. Parke-Houben, L. Hartmann, C.N. Ta, M.F. Toney, C.W. Frank, *Macromolecules* 2010, **43**, 6861-6870.
[8] F. Gobeaux, E. Belamie, G. Mosser, P. Davidson, P. Panine, M.-M. Giraud-Guille, *Langmuir* 2007, **23**, 6411-6417.