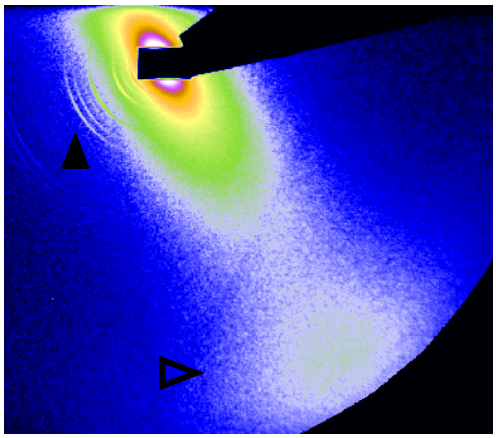
ESRF	**Experiment title:** ***Collagen fibrillar gels from cholesteric solutions, and association to a mineral phase.***	**Experiment number**: SC1934
Beamline: ID 02	**Date of experiment**: from: 19/07/2006 to: 21/07/2006	**Date of report**: 09/2006
Shifts: 6	**Local contact(s)**: Dr. Pierre Panine	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

E. Belamie*, Laboratoire de Chimie de la Matière Condensée, Equipe Matériaux du Vivant, UMR 7574, 12, rue Cuvier 75005 Paris, FRANCE
G. Mosser*, F. Gobeaux*, N. Nassif*, MM Giraud-Guille: same affiliation
Patrick Davidson*, Laboratoire de Physique du Solide, UMR 8502-CNRS Bat.510, Université Paris-Sud 91405 Orsay Cedex, FRANCE

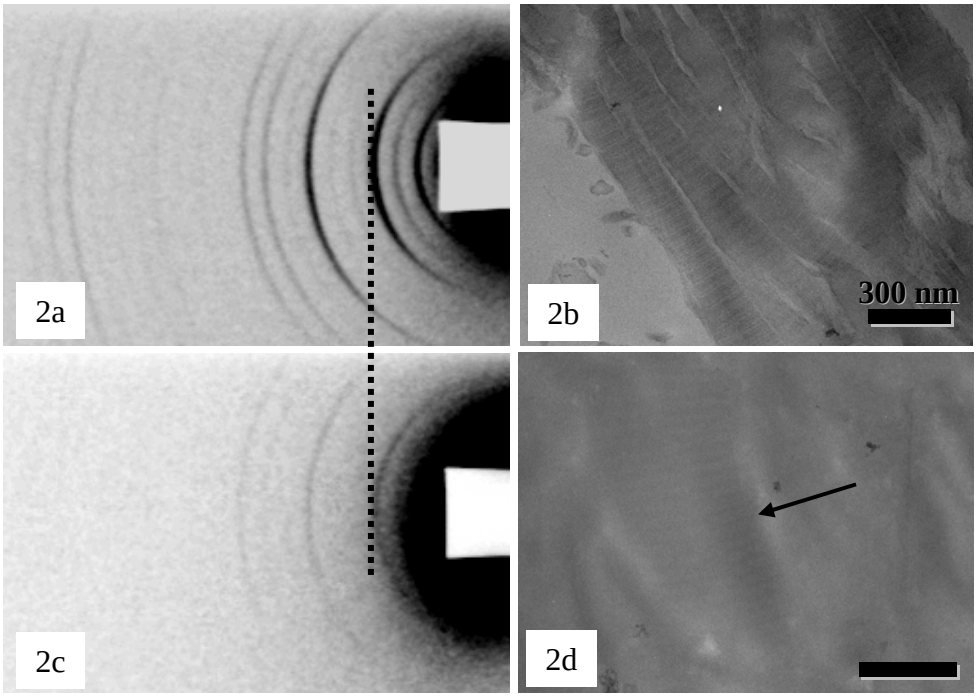
Report:

Collagen is one of the most abundant and ubiquitous protein of the animal kingdom, representing about 1/3 of all human proteins. In connective tissues, and in particular skeletal materials, it plays a considerable role as a structuring organic component to which a mineral phase of calcium phosphate is often closely associated. Type I collagen is for instance the principal protein forming the organic fibrous ordered network in bones, skin, ligaments, tendon and cornea.

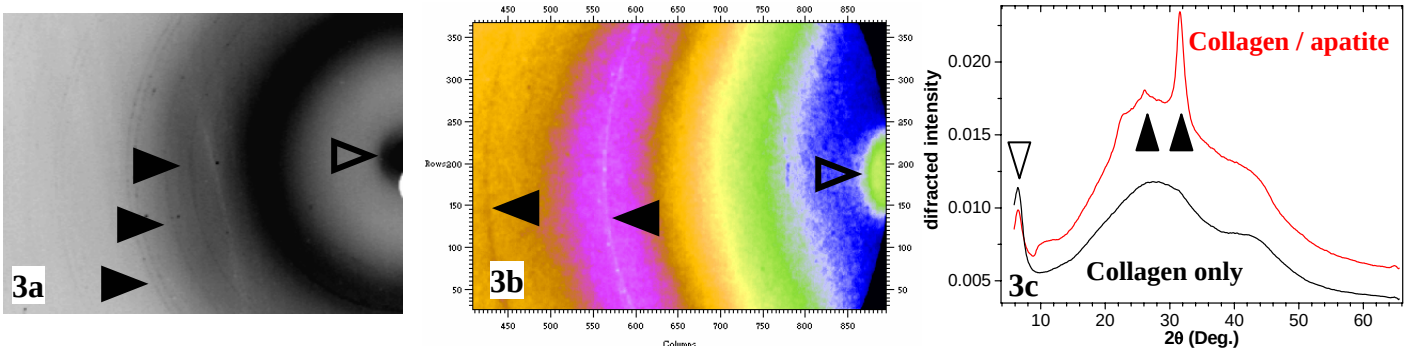


Our main objective during the run on ID02 in July 2006 was to study the formation of periodical structures in dense collagen solutions. These ordered aggregates called “fibrils” have been widely described in most of the major connective tissues cited above. Previous attempts to study fibrils formation were done in very dilute conditions, typically below 1 mg/mL and did not take into account the extreme molecular crowding characteristic of biological conditions. Collagen is secreted by specialized cells and concentrates in the narrow extracellular space between the cell membrane and the solid matrix present a few tens of microns above. Therefore, fibrillogenesis, the process by which collagen molecules neatly arrange themselves into periodic fibrils, occurs between macromolecules in a very dense state. We showed during a previous run on ID2 that collagen solutions undergo a phase transition from a disordered isotropic phase to a cholesteric one. The transition threshold is situated near 100 mg/mL, which is about its concentration in living tissues. We thus decided to investigate fibrillogenesis at high concentrations ranging from about 20 mg/mL up to 200 mg/mL in different conditions of pH and ionic strength to try and decipher the molecular mechanisms at play in such dense viscoelastic fluids. The SAXS pattern of figure 1 shows the typical features observed when a dense collagen solution at acidic pH, strongly shear aligned in a capillary tube, is brought to neutral pH by a switch in solvent. The black arrow head indicates the series of Bragg reflexions arising from the well-known 67 nm axial repeat. At higher q-values (open arrow head), the broad interference peak arising from lateral interactions between collagen molecules in the fibrils is visible near the edge of the SAXS detector. It was thus necessary to combine WAXS and SAXS detections so as to access the wide range of q-vectors necessary to study both signals in the concentration range of interest.

The diffraction signal corresponding to the 67 nm axial periodicity is very strong with samples kept at neutral pH, as clearly visible in figure 2a. TEM observations of similar samples confirm the presence of cross-striated fibrils. It must also be noted that the diffraction patterns are anisotropic due to shear-alignment of the collagen solution prior to the pH switch. Collagen has a charge close to zero around neutrality and forms fibrils at this pH because the repulsive interactions essentially cancel out.



At concentrations above 200 mg/mL and at acidic pH, the presence of fibrils is revealed by the apparition of the corresponding diffraction peaks (Figure 2c). Although weak, the peaks are clearly visible, at least the 4th, 6th and 9th orders of the 67nm periodicity. TEM observations also reveal the apparition of fibrils in these highly concentrated solutions as faint cross-striated structures (Figure 2d). It is the first time to our knowledge that this transition is observed. Given the structure described for the fibrils *in vivo*, the fibrils formed in highly concentrated acidic solutions of collagen are likely to be arranged as a slightly distorted hexagonal phase.



Strong fibrillar gels as described here are formed at concentrations close to that of connective tissues like skin, tendon or bone. In the latter case, collagen is associated to a mineral phase, a composite structure that it would be interesting to reproduce in the perspective of tissue engineering applications. Figure 3 shows diffraction patterns obtained with a dense collagen gel impregnated with calcium and phosphate ions; figure 3a was obtained with a WAXS CCD 2D detector and figure 3b was taken with an image plate. While at relatively low angle the interference peak between triple helices is visible close to the detectors' edge (open arrowhead), several Bragg diffraction rings corresponding to a mineral phase (black arrows) are clearly visible at larger q-vectors. The small angle diffraction patterns are anisotropic because shearing the collagen solution prior to forming the gel produces uniaxially aligned materials. Wide angle diffractograms do not appear strongly aligned, although the intensity variations of Bragg rings as a function of the azimuthal angle somewhat follow collagen orientation. As seen in TEM (Fig. 2b), fibrils are strongly aligned at the micron scale. Electron microscopy imaging and diffraction are currently underway to probe locally the interactions between the organic and mineral phases and to localize the nucleation sites. Typical peaks corresponding to the apatite phase (002: 25.9° and 211:31.8°) are visible on the upper profile in Figure 3c, which was obtained with a uniaxially aligned collagen gel soaked in a metastable solution of calcium and phospate ions. Further x-ray investigations will probably require the use of microbeam or nanofocus setups to be able to establish an orientational relationship between collagen and the calcium phosphate mineral phase.