ESRF	**Structural anisotropy characterization of fiber-reinforced hydrogels scaffolds during extrusion-based 3D printing for soft tissue repair**	**Experiment number**: MA-5550
Beamline: BM02	**Date of experiment**: from: 26-10-2022 to: 28-10-2022	**Date of report**: 17-08-2023
Shifts: 6	**Local contact(s)**: Isabelle Morfin	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

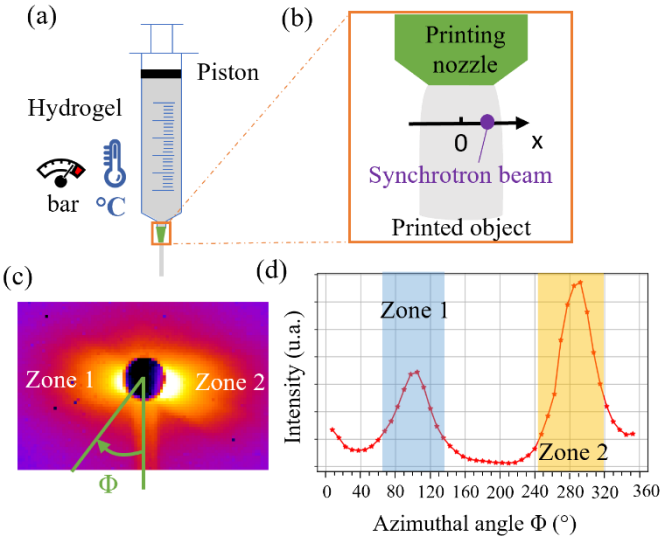

Fig. 1 (a) Experiment setup with the syringe filled with hydrogel precursor, driven by pressure, at a fixed temperature. (b) Definition of the relative positions *x* of synchrotron beam within the extrudate. (c) A SAXS diffraction image on the sample edge with a fixed *q*-value and a value of x/R = 1 where x is the relative beam position from the center of the extrudate and R is the printing nozzle radius (d) Cake plot of the intensity at a given q value where Zone 1 is the zone of interest for the orientation of the given hydrogel and Zone 2 corresponds to the reflection artefact on the surface of the extrudate.

- **Objective & expected results: -**

Bioprinting is the process where a tissue engineering construct is manufactured by bioink deposition. One possible bioprinting process is the controlled micro-extrusion from a cartridge through a nozzle of a hydrogel precursor solution, encapsulating cells or other biological bodies as displayed in **Fig. *1* (a)**. The applied pressure, the temperature and the geometry of the printing nozzle are defined as printing parameters. In this experiment, a bioprinting setup was installed to study the extrudates *in situ* with synchrotron beam. During the extrusion, the hydrogel precursor viscous solution is sheared yielding macromolecular orientation which depends on the printing parameters. The objective was to capture the SAXS and WAXS images across different positions in the extrudate (relative position *x*) *in situ* at the nozzle exit during extrusion of the viscous filaments. This enabled the orientation mapping of the macromolecular network (alginate, gelatin) and nanofiber filler like cellulose nanocrystals (CNF) introduced in the viscous system. The aim was to study *in situ* the effect of the printing parameters on the polymer formulation anisotropy, *i.e.* hydrogel constituent's orientation during extrusion.

- **Results and conclusions of the study: -**

Fig. 2 (a) displays typical X-ray diffraction diagrams of hydrogels (with water content ~90%), obtained after

radial average around the beam center, with water amorphous hallo and the crystalline contribution of the hydrogel formulation.

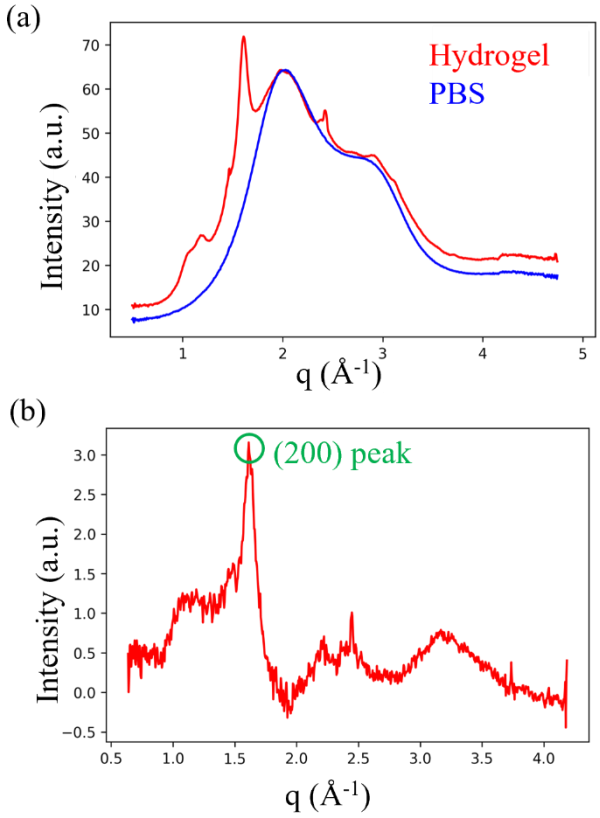

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Fig. 2 (a) Radial average diffraction pattern of the extruded hydrogel containing cellulose nanofiber- , bathed in phosphate buffer saline (PBS) (b) Extraction of the diffraction signal of the cellulose nanofibrils in which the (200) reflexion of cellulose is highlighted .

The anisotropy was evaluated by recovering the 2D scattered image at different positions along the extrudate. The intensity is then compared for different azimuthal angles ϕ for a given scattering vector value as schematized in the **Fig. *1* (d)**. For example, the orientation of cellulose crystals can be evaluated at the *q* value corresponding to the (200) reflexion as depicted in ***Fig. 2 (b)***. Such cake plot was created for each position of the extrudate, *i.e.* at each *x*-value. Close to the edge of the extrudate, the SAXS scattering displays an anisotropic orientation state, as evidenced in **Fig. *1* (d).** To quantify orientation, the apparent Herman's orientation factor[1] *f* was computed for each *x* position. The evolution of *f* with respect to the *x*-position values was obtained to map the orientation in the extrudate. In WAXS images, after subtraction of the water contribution, the diffraction pattern of CNF was obtained. As illustrated in **Fig. *2* (b),** this CNF pattern is easy to identify showing the sharp diffraction peak corresponding to the (200) family of planes. Consequently, the orientation map at the WAXS scale was only based on the orientation of cellulose crystals. For SAXS images, the *q*-value can be chosen to analyse the scattered intensity mainly due to the macromolecular network (alginate/gelatin/chitosan mix). Moreover, due to the reflection close to the surface, artefacts close to one edge of the sample needed to be considered (Zone 2 in ***Fig. 1 (d)***). As with WAXS, the evolution of *f* with respect to *x*-value was computed.

Our observations have demonstrated that (*i*) the anisotropy *i.e.* hydrogel constituent's orientation can be observed *in situ* through SAXS and WAXS measurements, and (*ii*) is influenced by the printing conditions, offering the perspective to manufacture anisotropic cellularized tissue engineering constructs with the use of 3D bioprinting. However, such orientation is only significant in the outer part of the extrudate where the highest shearing of the viscous ink is occurring, since at the center of the sample, the hydrogel is evidenced to be mainly isotropic. High shear conditions and a better control of the gelation point seem to be necessary to propagate orientation in the center of extrudates.

- **Justification and comments about the use of beam time: -**

The analysis of the effect of printing parameters on the orientation of 3D printed extrudates *in situ* during extrusion was made possible by combining SAXS and WAXS (With WOS detector). The results will be submitted for publications.

References:

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