ESRF	**Experiment title: Development of cellulosic based composites with typical three-layer orthotropic cartilage organization by combined ultrafiltration, ultrasound and UV photopolymerization.**	**Experiment number:** SC-5319
Beamline: ID02	**Date of experiment:** from: 14/02/2023 to: 16/02/2023	**Date of report:** 01/09/2023
Shifts: 9	**Local contact(s): W. Chèvremont**	*Received at ESRF:*

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Report: The focus of this project is to study *in-situ* the combined effect of shear flow, pressure and ultrasound waves on the structural organization of colloidal suspensions with the aim of developing novel tunable nano- to micro- structured materials. Accessing a wide range of length scales, subnanometric to a few micrometers will shed light on the orientation mechanisms and structural organizations induced by the relative intensity of these external forces.

Recently, we were able to evidence the ability of the ultrafiltration processes to develop well-defined layered structures of anisometric colloidal particles aligned horizontally along the velocity direction from nanometer to micrometer length scales [1-3]. Furthermore, in our last proposal SC-5110, novel processing methods combining frontal ultrafiltration (FU) under ultrasounds (US) applied on nanocrystal celluloses (CNCs) aqueous suspensions, have allowed to achieve the three orthotropic structured layers representatives of the multizonal material cartilage [4].

Time resolved in-situ SAXS combined with dedicated channel-type FU/US cell, revealed a parallel orientation of the CNCs near the membrane surface (superficial zone), an intermediate isotropic layer (middle zone) and a vertical orientation layer of the CNCs near the ultrasonic vibrating blade (deep zone) (Fig.1). Nevertheless, after cessation of transmembrane pressure, relaxation processes have been noticed, and the three-layered organization or the anisotropic layered structures were lost. Consequently, one way to freeze the orientations is to add a UV cured polymer to the filtered colloidal suspension and apply in-situ photopolymerization at the end ofthe builded multilayered organization by FU/US processing.

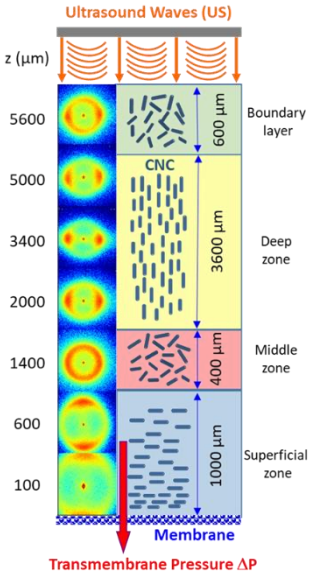

Fig. 1: Typical orthotropic organization of cartilage achieved by FU under US. 2D-SAXS patterns at t = 88 min.

The goal of this proposal was firstly to use in-situ SAXS, to bring an understanding of the organization mechanism induces during FU/US-UV curing processing and secondly, to characterize solid cellulosic UV cured nanocomposites after different FU/US conditions.

First result: in-situ characterization of the CNCs aqueous suspensions organizations during FU/US Process.

A dedicated SAXS channel-type ultrafiltration/ultrasound cell was implemented at ID02 to simultaneously generate an US-induced vertical acoustic radiation force at the top of the channel and simultaneously a transmembrane pressure force at the bottom of the channel near the membrane surface. The SAXS patterns where regularly registered as a function of the distance z from the membrane surface and as a function of FU/US time processing. Different US Power were explored at two different initial CNCs concentrations.

Thanks to this set-up and in situ SAXS, and according to first experiment on precedent run SC-5110, the typical multilayered orthotropic structuring that mimic the articular cartilage organization was confirmed for two different CNCs initial concentrations and for different ultrasound acoustic powers from 20 to 100 %.

In this run, the ultrasound power was modified and we explored the effect of this US acoustic power on the organization and thickness of the three differently structured zones. By using the values of the PCA anisotropy parameter (calculated using principal component analysis (PCA) in SASET software) for different US power conditions, it was possible to determine the thickness of each of the different oriented layers (Fig. 2). It appears that the lower horizontal layer is not affected by the ultrasonic power because it results from frontal ultrafiltration. The variation in thickness is therefore linked to the different filtration time between each ultrasound power. On the other hand, the further one moves away from the membrane surface, the greater the effect of the ultrasonic radiation forces, leading to a change in the orientation of the CNCs. It can be seen that the thickness of the top two layers varies as a function of ultrasound power, whatever the CNC concentration.

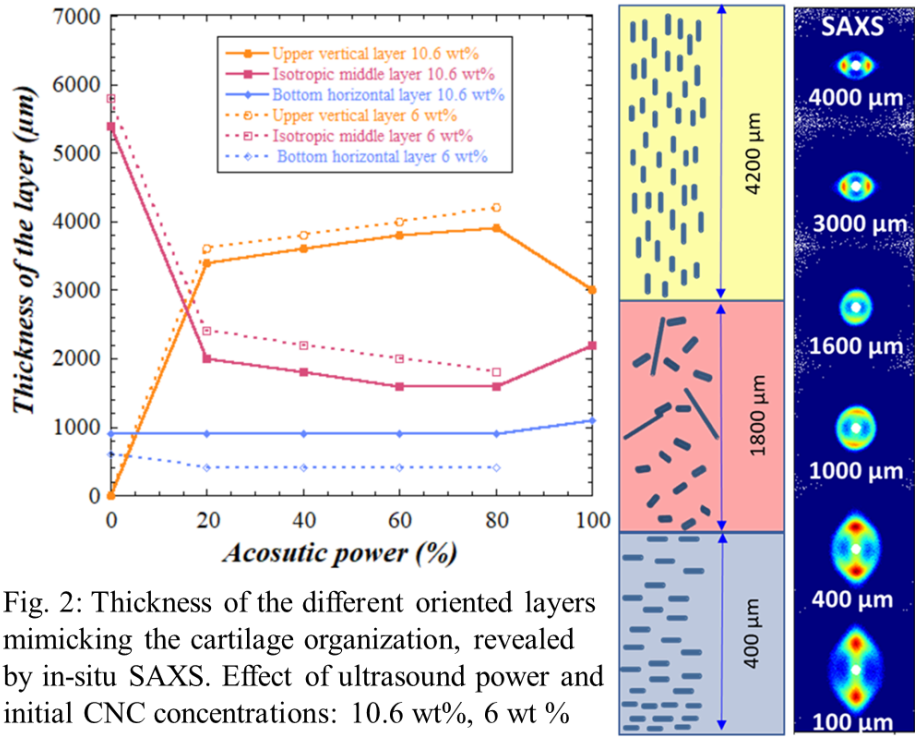

Fig. 2: Thickness of the different oriented layers mimicking the cartilage organization, revealed by in-situ SAXS. Effect of ultrasound power and initial CNC concentrations: 10.6 wt%, 6 wt %

Second result: in-situ characterization of the solid CNC/PEGDA nanocomposites obtained by FU/US followed by UV curing process. First evidence of ability to freeze the three-layered mimicking the cartilage organization.

A second experiment was carried out using light-cured solid nanocomposites (PEGDA/CNC) previously prepared in the laboratory. Precise scans were taken at 25 µm intervals within these nanocomposites. Thanks to this, a first promising result could be shown with the fixed orthotropic structure (Fig. 3). This structure consists of three well-defined orientation zones: a first layer located close to the membrane with a thickness of 150 µm, where the CNCs are oriented parallel to the membrane; a second layer of 475 µm with an isotropic organisation; finally, a third layer of 4750 µm where the CNCs are oriented vertically.

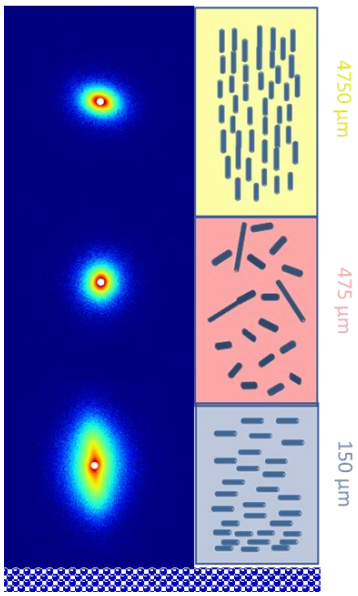

Fig. 3: Solid nanocomposite obtained by FU/US process during 2 h at 1.2 x 10^5 Pa transmembrane pressure and 100 % US acoustic power, followed by UV-curing. SAXS patterns at different height in the sample, revealing the three sub-layer mimicking the cartilage organization with (parallel, isotropic and perpendicular) orientation of the CNCs from the reference membrane surface. Initial suspension: 65/35 PEGDA/CNC with 5 wt% CNC concentration.

Third result: in-situ characterization of the solid CNC/PEGDA nanocomposites obtained by only FU followed by UV curing process. Effect of filtration time and relaxation time before UV-Curing on the densification and anisotropic organization of the CNCs.

Finally, nanocomposites manufactured by frontal filtration and subsequently photopolymerised were analyzed. A concentrated layer with a structure of CNC layers oriented parallel to the membrane was clearly observed and quantified. In addition, various process parameters were explored, such as filtration time, concentration and relaxation time prior to photopolymerization.

It was shown that it was possible to control the gradient of the CNCs interparticle distance d as well as the thickness of the concentrated deposit near the membrane surface by tunning these parameters. For example, in Figure 4, it can be seen that the CNCs interparticle distance d decrease at increasing filtration times, in accordance with denser, thicker and more concentrated layers near the membrane surface. The corresponding PCA anisotropy increase also with the same distribution gradient of CNCs interparticle distance d as a function of the distance z from the membrane surface. These results emphasis the success of this new frontal filtration and photopolymerized process, to reach dense and higly anisotropic cellulose nanocomposites.

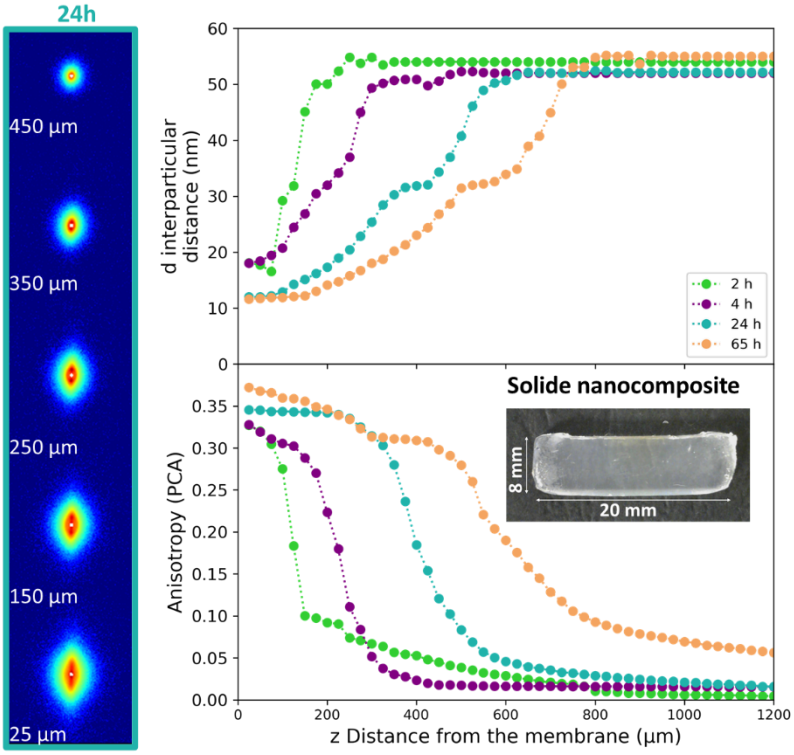

Fig. 4: Influence of filtration time on the interparticle distance and PCA anisotropy profiles of nanocomposites (PEGDA/CNC 2 wt %) and example of corresponding SAXS patterns at different heights in the sample.

The next step will be to characterize the mechanical strength and corresponding change in structural organization of these cellulose nanocomposites, using in-situ SAXS extension/compresion tests.

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