Experimental report

19/02/2019

Proposal: 9-13-778 **Council:** 4/2018

Title: Gelation and aggregation behavior of green composit hydrogels derivedfrom regenerated silk fibroin

Research area: Materials

This proposal is a new proposal

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Samples: Silk Fibroin
Sophorolipids

Instrument	Requested days	Allocated days	From	To
D11	0	2	10/10/2018	12/10/2018
D22	4	0		

Abstract:

Silk fibroin (SF) is a biocompatible and –degradable material that has raised interest for applications in the biomedical field. SF is regenerated from the cocoon of the silkworm in a degumming and dissolution process, which often degrades the protein and alters its properties. Here we want to study novel hybrid silk fibroin based gels that use regenerated SF in its native form. We combine these gels with Sophorolipids (SL) to form a green composite hydrogel. SL are microbially produced glycolipids with anti-microbial, anti-inflammatory and anti-cancer properties. Micellar SL were reported to form hydrogels with fragmented SF with adjustable pore sizes and application as 3D scaffolds. We want to combine SL in their micellar and non-micellar form with native SF to join their inherent biomedical properties.

Standard Project

Experimental Report template

Proposal title: Gelation and aggregation behavior of green composit hydrogels derivedfrom		**Proposal number: 9-13-778**
Beamline: D11	**Date(s) of experiment:** from: 10/10/2018 to: 11/10/2018	**Date of report:** 14/02/2018
Shifts:	**Local contact(s):** Anne Martel	*Date of submission:*

Background: The protein silk fibroin (SF), produced by the *B. mori* silkworm, is an interesting material with exceptional mechanical properties paired with inherent biocompatibility and degradability. SF consists of a heavy (FibH, ~390 kDa) and a light chain (FibL, ~26 kDA), held together by a disulfide bond. For biomedical applications SF is regenerated from the cocoon of the silkworm in a degumming and dissolution process. In all current literature however, inappropriate protocols for the preparation of regenerated SF (regSF) are applied which degrade the FibH part, visible in very broad size distributions, influencing the properties of SF, especially the gelation behavior. RegSF gelates upon a conformational change of FibH from random coil to β-sheet. The gelation process can be influenced by addition of MeOH and salts, pH, temperature and mechanical shear. SF gels have tunable mechanical properties, paired with biocompatibility and ease of fabrication and have thus been investigated as an ideal matrix for cell culture, tissue engineering and wound dressing. We want to study **novel hybrid silk fibroin based gels** that use **regenerated SF in its native form**. We combine these gels with **Sophorolipids (SL)** to form a **green composite hydrogel**. SL are microbially produced **glycolipids** with anti-microbial, anti-inflammatory and anti-cancer properties. SL were reported to form hydrogels with SF with adjustable pore sizes and application as 3D scaffolds. In this study, SL in the **micellar form** were used as a gelator for **fragmented SF** to speed up the gelation time and the effect was attributed to the structure of SL. However, we believe that the chosen q-range ($0.01 < q$ [Å-1] < 0.3) in this study was not wide enough to cover the full range of structure of SF gels and the application of 100 % D2O as solvent might have altered the aggregation behavior. We have previously investigated the structure of SL by SANS (see preliminary work section) and want to **combine them in their micellar and non-micellar form with native SF** to join their inherent biomedical properties.

Results and the conclusions of the study (main part):

The experiments have been done with a wavelength of 5.3 Angstrom, sample-to-detector distances 1.4, 8 and 39 m as well as a wavelenght of 13.5 Å at 39 m sample-to-detector distance to reach very low q. Neutron absorber was B4C and Hellma 1 mm cells have been used for all samples. Each sample was acquired for 1 minute up to 1h for weakly scattering samples.

We analyzed different preparations of silk fibroin with sophorolipids in different D_2O and H_2O mixtures. A first and important point was to see whether D_2O alters the structure of silk fibroin. We concluded that the change in struccture is only a kinetic one but not a structural (Figure 1A, table 1). Data were fitted with SASview with a model for an elliptical cylinder. The extracted data revealed that silk fibroin changes from more spherical objects to elongated fibers upon gelation.

	χ^2/Npts	r_{minor} / nm	axis ratio	length / nm
SF fresh H_2O	1.18	2.0	4.4	150
SF gelled H_2O	1.33	2.1	3.9	1700
SF fresh D_2O	1.54	1.9	4.7	1100
SF gelled D_2O	2.66	2.1	4.6	2000

In a second step we analyzed different fibroin-sophorolipid mixtures at different SL concentrations. Figure 1B shows a representative example of such a composite hydrogel (red line). All samples were measured in D_2O, H_2O and at 50 % D_2O which is the matchpoint of fibroin. We could show that the two compounds do not interact with each other but rather form an orthogonallly-assembled system in which each compound keeps its initial structure. This could be revealed by subtracting the curve of SL only (Figure 1B, black curve) from the data of the hybrid hydrogel. Plotting the resulting data (Figure 1C, cyan) together with the data for fibroin only results in very similar curves, hypothesizing that there is no intercalation of SL with SF. The extracted parameters from the fit (elliptical cylinder) showed that the fibers formed in the SL-SF hydrogel are slightly longer that those for SF only. However, analysis is still ongoing and the fits have to be optimized. Similar measurements were done with other sophoro- and glycolipids, showing similar results. Analysis of this data is still ongoing.

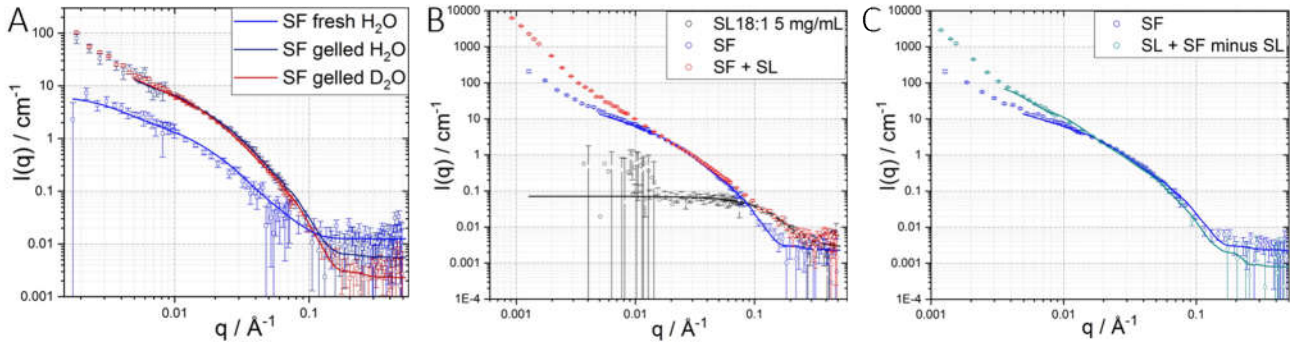

Figure 1. SANS curves for A) silk fibroin in H2O not gelled (bright blue), SF in H2O gelled (dark blue)and SF in D2O (red, superimposed with dark blue), B) SANS profile for a hybrid hydrogel of SF and SL (red), SF only (blue and SL only (black), C) SANS data for the composite gel withsubtracted SANS data for the SL only (cyan) and SANS for SF only (blue)

Data treatment

All samples have been treated at the beamline soon after acquisition. The CCD images have been integrated and correction for absolute calibration was done using the correction factor for water (0.9). Background (cell + appropriate solvent) has been measured and subtracted from the data. Incoherent scattering was subtracted when necessary.

Justification and comments about the use of beam time:

The use of the neutrons was necessary because of the complexity of the system and the fact that the main components were organic with low contrast to X-rays. One of the compounds, silk fibroin, that is a protein is prone to denaturation when iradiated with x-rays. The use of D11 was ideal to cover the large required q range.

Problems during beamtime:

We did not experience any trouble during the beamtime.