

# Modulating Intermolecular Interactions and Rheological Properties in Silk Fibroin Using Ultrasound Frequencies

Serôdio R<sup>1</sup>, C Cunha-Reis<sup>1</sup>, Oliveira AL<sup>1\*</sup>

<sup>1</sup> CBQF - Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal

\*corresponding author: aoliveira@porto.ucp.pt



CATOLICA  
FACULTY  
OF BIOTECHNOLOGY

PORTO

## Introduction/Resume

Techniques such as electrospinning are often used in the production of nanofiber scaffolds that mimic the nanostructure of native extracellular matrix. [1] Fiber formation from a polymer solution is primarily governed by the entanglement of polymer chains and it occurs when an increase in viscosity assures that the overlap concentration is reached [2]. Hence, the viscosity of the starting solution is amongst the most important parameters to achieve optimal processing conditions and fiber quality. Silk fibroin (SF) is a self-assembling structural protein in natural silkworm fibers, that exhibits excellent mechanical properties, biocompatibility and inducible formation of crystalline  $\beta$ -sheet structure networks, that regulate the rheological properties of SF solutions.

Solutions of regenerated silk are often obtained with very low viscosities. This parameter is usually controlled either by manipulating the degumming time in order to achieve a higher molecular weight [2] or by concentration against water soluble polymers such as Polyethylene glycol (PEG) [3]. Both of these methods consume time and resources. In this study we investigate the effect of ultrasound sonication on the intermolecular interactions and transition to  $\beta$ -sheet conformation of SF aqueous solutions, as a strategy to physically enhance the rheological properties of SF solutions and improve the system fiber-forming ability.

## Materials & Methods

SF from Bombyx Mori cocoons was used to prepare aqueous solutions (7 wt%).

Solutions were exposed to direct sonication (70Hz) for controlled periods of 120 up to 330s.

Instantaneous viscosity was analyzed by oscillatory sweep assay from 0.1 to 10 s<sup>-1</sup> shear rate. The maximum viscosity was considered.

Solutions were electrospun with an 30kV voltage and 20  $\mu$ L/s flow rate.

Fourier Transform Infrared (FTIR) analysis were performed immediately after sonication and in subsequent time points up to 4 hours of settling time.

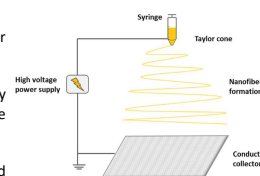


Figure 1. Diagram of the electrospinning apparatus.

## Results

### Rheological Analysis

The influence of sonication time on the rheological properties of the solution was studied. Controlled sonication periods induced the gelation of the SF solutions, and therefore increased the viscosity. Moreover, sonication triggers self-assembly phenomena that were observed over the range of settling times under study.

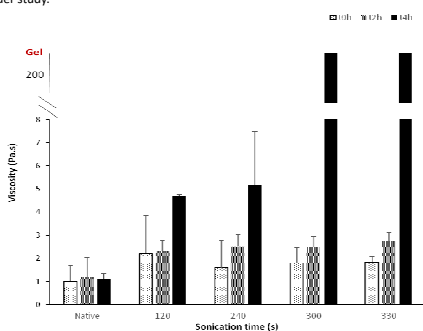


Figure 2. Maximum viscosity as function of sonication time (s).

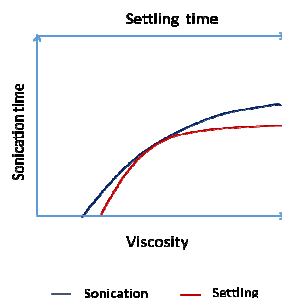


Figure 3. Influence of sonication and settling times over the viscosity.

### FTIR Analysis

SF underwent conformational transitions that translated into the formation of crystalline  $\beta$ -sheet structure networks, confirmed by the observation of a shoulder at the Amide I region, in the sonicated samples.

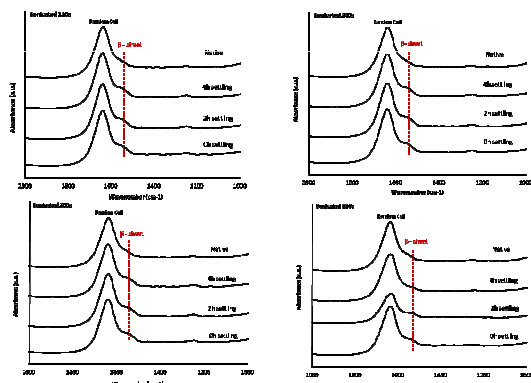


Figure 5. The increase in sonication and settling periods led to the emergence of a band at the 1500-1600 cm<sup>-1</sup> region, indicative of conformational transitions towards  $\beta$ -sheet formation.

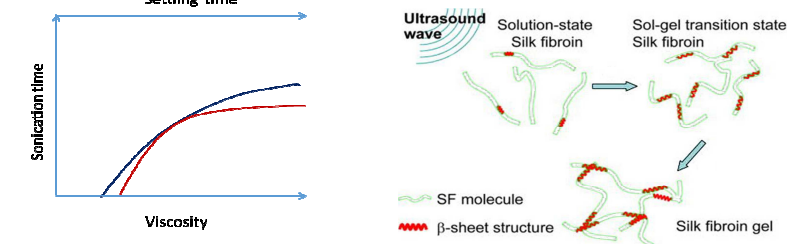


Figure 4. Effect of ultrasound frequencies on SF gelation. The gelation process contains two kinetic steps: a, structural change from random coil to  $\beta$ -sheet with some inter-chain physical crosslinks occurring in a short time frame; b,  $\beta$ -sheet structure extended, large quantity of inter-chain  $\beta$ -sheet crosslinks formed, and molecules organized to gel network over a relatively long time frame. Adapted from [4].

### Electrospinning

SF solutions with viscosities ranging 1-10 Pa.s were obtained by exposure to ultrasound sonication. The viscosity of the solutions was increased by either using longer sonication periods, or by using shorter sonication periods and extending the settling time. Hence, equivalent viscosity values were obtained by using different combinations of both parameters. Using low viscosity solutions (1-3 Pa.s), fiber formation was not observed. Better spinnability was achieved when the viscosity of the solutions showed values ranging from 3-5 Pa.s. However bead-like fiber morphology was observed (a). Increasing the viscosity (5-10 Pa.s) drastically enhanced the spinnability of the solutions, and therefore leading to the production of nanofibers with adequate morphology.

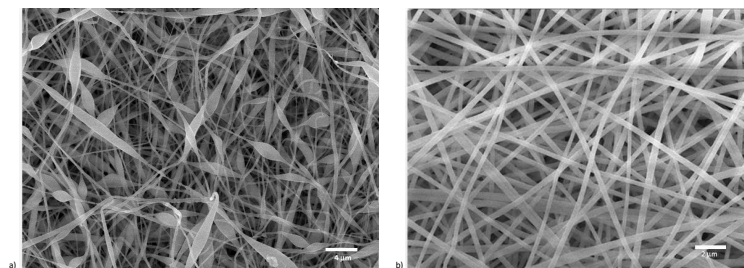


Figure 6. Representative SEM micrographs of electrospun nanofibers. a) Intermediate viscosity (< 3 Pa.s) and b) High viscosity (> 3 Pa.s)

## Conclusions

The present study constitutes a step forward towards the understanding and fine tuning of intermolecular interactions in silk fibroin solutions prior and after electrospinning. A simple sonication treatment is able not only to induce the immediate formation of  $\beta$ -sheets, but also to trigger the self-assembly of these structures over sustained periods of time after sonication, leading to a marked increase in the viscosity of the solution. The application of this processing strategy enables the achievement of optimal processing conditions that lead to high-quality electrospun nanofibers.

### References

- [1] J.L. Daristotle et al., ACS Appl Mater Interfaces, 28, 8 (51), 34951-34963 (2016)
- [2] B.P. Partlow et al., Macromolecular Bioscience, 16, 5, 666-75 (2016)
- [3] Y. Kishimoto et al., Mater. Sci. Eng. C 73, 498-506 (2017)
- [4] W. Xiaojin et al., Biomaterials 29.8 (2008): 1054-1064, (2017).

### Acknowledgements

"IBEROS" 0245\_IBEROS\_1\_E, financed by Programme INTERREG V-A Spain-Portugal 2014-2020 (POCTEP). "Biotherapies- Bioengineered Therapies for Infectious Diseases and Tissue Regeneration", funded by Norte2020. A.L. Oliveira greatly acknowledges the Portuguese Foundation for Science and Technology, for the Investigator FCT program IF/00411/2013, project SERICAMED (IF/00411/2013/CP1167).