Silk Fibroin Aerogel Particles for Wound Healing Treatment

Beatriz G. Bernardes[1,2], Sara Baptita-Silva[1], Carlos Illanes-Bordomás[2], Rui Magalhães[1], Raquel Costa*[3,4,5], Carlos A. García-González*[2], Ana Leite Oliveira*[1]

1 Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal
2 Department of Pharmacology, Pharmacy and Pharmaceutical Technology, I+D Farma group (GI-1645), iMATUS and Health Research Institute of Santiago de Compostela (IDIS), Universidade de Santiago de Compostela, E-15782 Santiago de Compostela, Spain
3 Instituto de Investigação e Inovação em Saúde, Universidade do Porto (i3S), Porto, Portugal
4 Department of Biomedicine, Biochemistry Unit, Faculdade de Medicina, Universidade do Porto, Porto, Portugal
5 Escola Superior de Saúde, Instituto Politécnico do Porto, Porto, Portugal
*raquel.costa@i3s.up.pt; carlos.garcia@usc.es; aloliveira@ucp.pt

Introduction/Resume

The healing process of an injury comprises a series of steps (haemostasis, inflammation and proliferation/maturation). Exudate from wounds is a natural response to heal. However, an excess production can compromise and delay the inflammatory phase, resulting in chronicity.
Novel biocompatible, biodegradable and adaptable dressings are sought to promote tissue regeneration, prevent infection and control inflammation. Aerogels are nanostructured dry materials with high porosity, large surface and low bulk density. Bio-based aerogels, from natural polymer sources, can provide advanced performance for wound healing; also, they can act as carriers for bioactive compounds.
Silk fibroin (SF) aerogels can act as promising carriers of bioactive molecules while supporting cell proliferation. Hereupon, SF aerogels were developed in the form of particles for wound healing applications, using supercritical CO2 technology.

Methods

Silk fibroin extracted from Bombyx mori cocoons was used to prepare SF aerogel particles. For the aerogel particles' production, SF aqueous solutions at different concentrations (3, 5 and 7 %(w/v)) were introduced into an absolute ethanol and Span 80 (3 wt.% with respect to SF) solution, followed by supercritical CO2 drying (120 bar, 39ºC, 3.5 h) (Figure 1). Ethanol was added at a ratio of 2:1(v/v) in relation to SF solution.

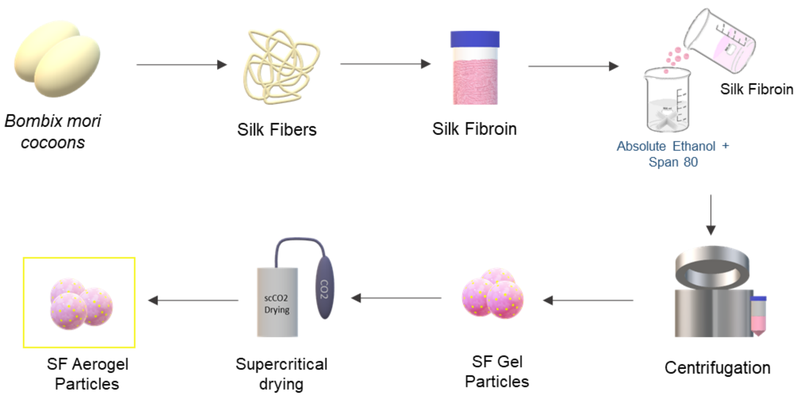

Figure 1 – Silk-based aerogel particles production method.

Chemical and structural characterization
- Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR)

Morphological characterization
- N2 adsorption-desorption tests
- Helium Pycnometer
- Laser Diffraction

Biocompatibility
- Evaluated by direct contact with Human Dermal Fibroblasts (HDF's) and observed by Scanning Electron Microscope (SEM). Quantitative data were subjected to an analysis of variance (one-way ANOVA, Tukey's test; α=0.05.

Results

The average diameter obtained on laser diffraction shows us that the concentration of SF influences the diameter gel particles and dispersion increased with increasing SF concentration (Figure 2).

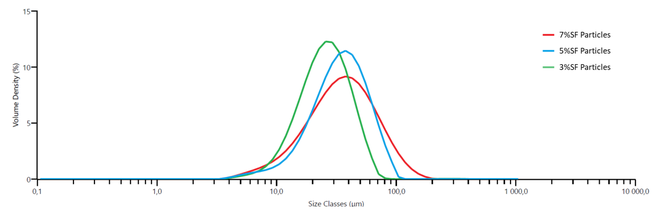

Figure 2 – Diameter dispersion of Silk-based aerogel particles

According to the FTIR-ATR analysis, it was possible to verify the presence of the main characteristic bands of SF assigned to the presence of β-sheet structure, characterized by strong bands on the amide I and II regions (Figure 3).

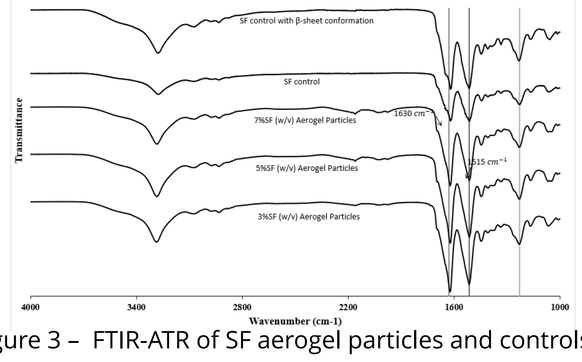

Figure 3 – FTIR-ATR of SF aerogel particles and controls .

Cell viability of SF Aerogel particles were tested using HDF's cell line. After 24 h of incubation, all the aerogels presented a cell viability of 50% and there were significant differences between the cells in contact with aerogel particles and the control group (Figure 4a). After 7 days of incubation, it was possible to verify that cell viability is higher than in the control, thus indicating that aerogel particles promote cell proliferation. These results were confirmed by SEM analysis (Figure 4b).

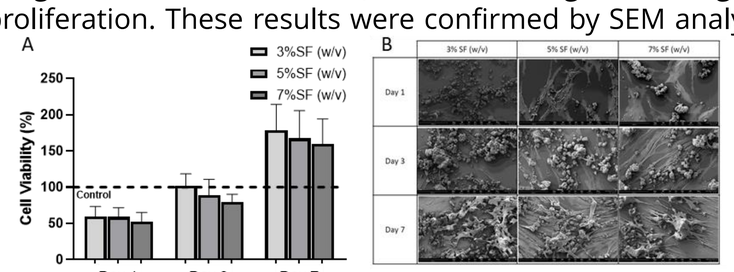

Figure 4 –A. Cell viability after MTT assay of HDF's cells in contact with aerogel particles as compared with the control group ($\alpha < 0.05$). B. SEM micrographs of HDF's cell cultures in contact with SF aerogel particles for 1, 3, and 7 days.

Conclusions

Physicochemical and textural characterization of the SF aerogels showed excellent properties, such as high biocompatibility and high surface area. Thus, this method is suitable for the production of particles for wound healing applications.

Acknowledgements
This research was funded by MICINN [PID2020-120010RB-I00], Xunta de Galicia [ED431C 2020/17], Agencia Estatal de Investigación [AEI] and FEDER funds. This work was also supported by National Funds from Fundação para a Ciência e a Tecnologia (FCT), through project UID/Multi/50016/2020, Doctoral Research Grant 2021.05717.BD and Post Doctoral research grant SFRH/BPD/116024/2016. Work carried out in the framework of the COST Action CA18125 "Advanced Engineering and Research of aeroGels for Environment and Life Sciences" (AERoGELS), funded by the European Commission; and project TEX4WOUNDS (POCI-01-0247-FEDER-047029), financed under the Incentive System for Research and Technological Development, R&DT Projects in co-promotion (Notice SI/17/2019).

[RYC2014-15239]/ [RTI2018-094131-A-I00] [ED431C 2020/17]