Supercritical CO_2 Technology for the Fabrication of Silk Fibroin Aerogel Particles for Wound Healing and Regeneration

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Introduction/Resume

The healing process of an injury comprises a series of steps (haemostasis, inflammation and proliferation/maturation). In wounds, the exudate is a natural response during healing. However, overproduction 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. Aerogels, from natural polymer sources, i.e., bio-based aerogels, can provide advanced performance for wound healing; also, they can act as carriers for bioactive compounds. [1]
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 CO_2 technology.

Methods

Silk fibroin solution extracted from *Bombyx mori* cocoons was used as aerogel source. For the aerogel particles' production, different SF aqueous solutions (i.e.: 3, 5 and 7% (w/v)) were mixed with absolute ethanol and Span 80 (3 wt.% with respect to SF), followed by supercritical CO2 drying (120 bar, 39ºC, 3.5 h).

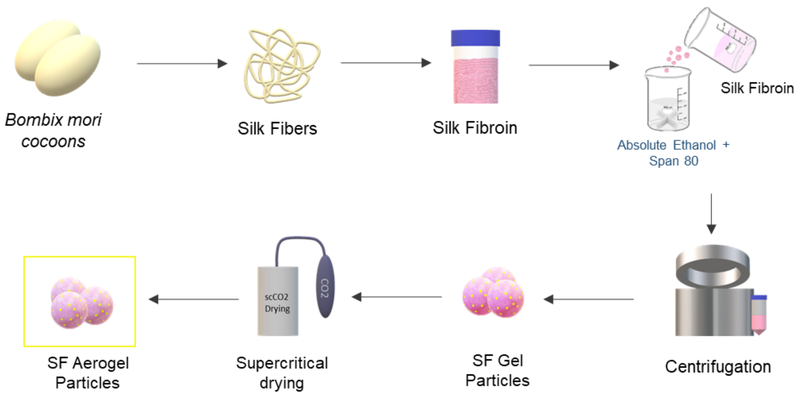

Figure 1 – Silk-based aerogel particles production method.

Chemical and structural characterization

- Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR).

Morphological and textural characterization

- Nitrogen adsorption-desorption tests
  - The Brunauer-Emmet-Teller (BET) method was applied to calculate the specific surface area (aBET). The specific mesopore volume (Vmes) was obtained from the cumulative Barrett-Joyner-Halenda (BJH) method-pore volume profiles of the aerogels in the mesopore range (2–50 nm). The specific volume occupied by the macropores (Vmac) in the aerogels was calculated as the difference between the total specific pore volumes of the aerogels and the Vmes. [2]
- Laser Diffraction with Ethanol 96% as dispersant medium.

Biocompatibility

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

Results

Figure 2 shows SEM micrographs of SF the Aerogel particles. Table 1 shows the textural properties of SF Aerogel particles (aBET, Vmes and Vmac)

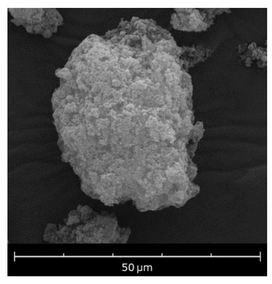

Figure 2 - SEM micrographs of Silk-based aerogel particles.

Particles	aBET (m^2/g)	Vmes (cm^3/g)	Vmac (cm^3/g)
3%SF	236 ± 11.84	1.42 ± 0.33	13.05 ± 0.33
5%SF	408 ± 20.41	2.21 ± 0.81	10.96 ± 0.81
7%SF	203 ± 10.17	1.16 ± 0.26	11.32 ± 0.26

Table 1 - Textural properties of SF Aerogel particles.

According to the FTIR-ATR analysis, it was possible to confirm the presence of the main characteristic bands of SF assigned the amide I and II regions. The presence of β-sheet conformation was verified by the position of amide II band (Figure 3).

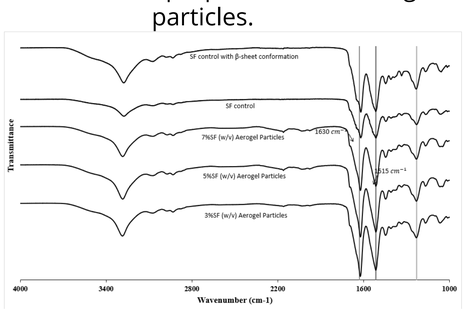

Figure 3 – FTIR-ATR of SF aerogel particles and controls.

Cell viability of SF Aerogel particles was tested using a 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 3 days of incubation, it was possible to verify that the cell viability strongly increased and after 7 days it was higher than the control, thus indicating that the aerogel particles promote cell proliferation. These results were confirmed by SEM analysis (Figure 4B).

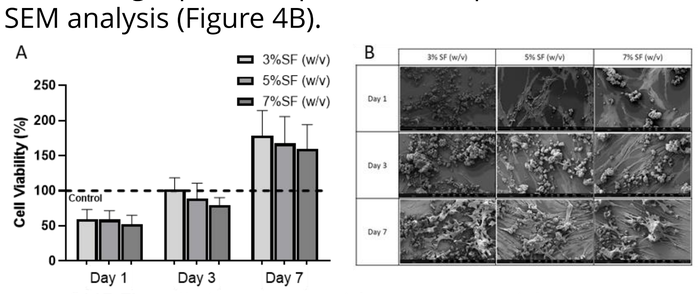

Figure 4 –A. MTT assay of HDF's cells in contact with aerogel particles as compared with the control group ($\alpha < 0.05$) for 1, 3, and 7 days.. B. SEM micrographs of HDF's cell cultures in contact with SF aerogel the particles.

Conclusions

SF particles showed excellent properties, such as high biocompatibility, high surface area and low skeletal density, suggesting that the aerogel technology is suitable to produce particles for wound healing applications. Confocal Microscopy, DNA quantification, antioxidant and degradation tests are currently on-going. These particles will be further studied as a promising drug delivery platform for wound healing applications.

Bibliography

1. Bernardes, B.G.; Del Gaudio, P.; Alves, P.; Costa, R.; García-Gonzaléz, C.A.; Oliveira, A.L. Bioaerogels: Promising Nanostructured Materials in Fluid Management, Healing and Regeneration of Wounds. Molecules 2021, 26, 3834. https://doi.org/10.3390/molecules26133834
2. López-Iglesias, C.; Barros, J.; Ardao, I.; Monteiro, F. J.; Alvarez-Lorenzo, C.; Gómez-Amoza, J. L.; García-González, C. A. Vancomycin-loaded chitosan aerogel particles for chronic wound applications. 2019 Carbohydrate polymers, 204, 223–231. https://doi.org/10.1016/j.carbpol.2018.10.012

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