Adenosine-loaded Silk Fibroin Aerogel Particles for Wound Healing

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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.[1] Adenosine (ADO) is a nucleoside that is expected to trigger the healing process of chronic wounds, promoting angiogenesis and regeneration.[2]

Silk fibroin (SF) aerogels can act as promising carriers of bioactive molecules while supporting cell proliferation. Hereupon, SF aerogels loaded with Adenosine were developed in the form of particles for wound healing applications, using supercritical CO2 technology.

Methods

For the aerogel particles' production, SF aqueous solutions at different concentrations (3, 5 and 7 %(w/v)) loaded with ADO at different ratios 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).

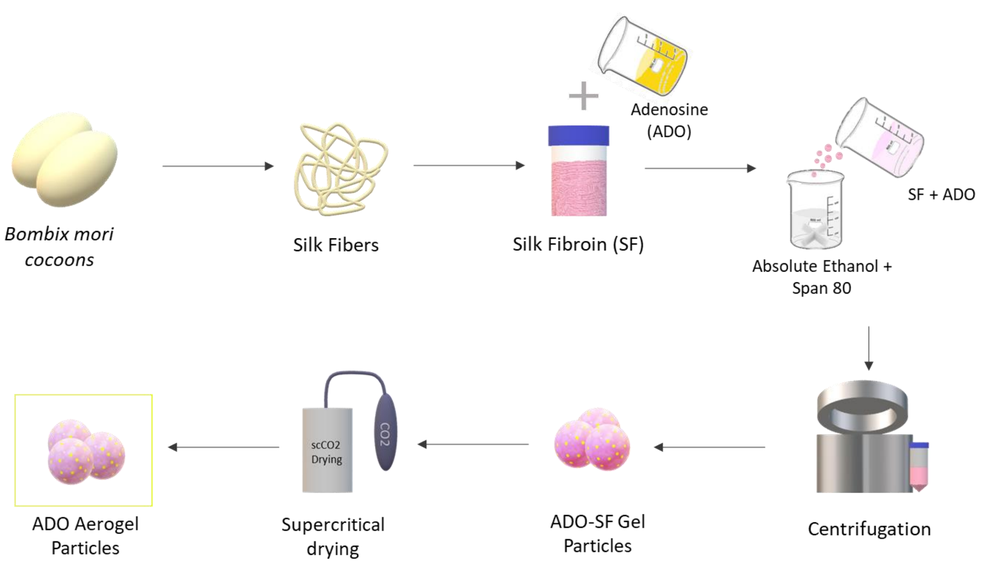

Figure 1 – Silk-based aerogel particles production method.

Chemical and structural characterization

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

Morphological characterization

- Laser Diffraction
- Scanning Electron Microscope (SEM)

Biocompatibility

- SF Aerogel particles unloaded with adenosine were firstly studied to evaluate their biocompatibility by direct contact with Human Dermal Fibroblasts (HDF's) and observed by SEM. Quantitative data were subjected to an analysis of variance (one-way ANOVA, Tukey's test; α=0.05.

Results

Laser diffraction shows us that the concentration of SF and ADO influences the gel particles diameter. Adding ADO in SF solution increased the viscosity of the solution, mainly on 7%SF particles. Consequently, in order to decrease dispersion an increase in ethanol volume for particles production was applied (Figure 2).

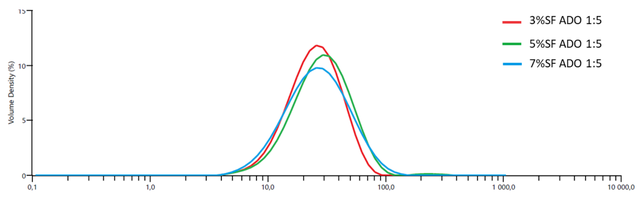

Figure 2 – Diameter dispersion of Silk-based aerogel particles.

Figure 3 shows the. SEM micrographs of SF Aerogel particles.

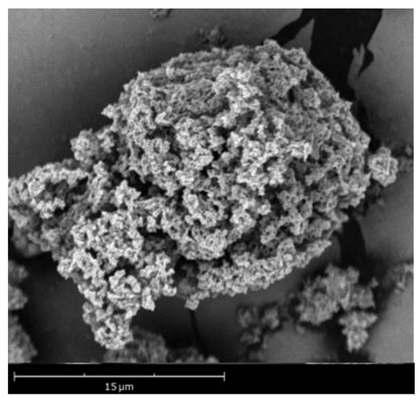

Figure 3 – SEM micrographs of Silk-based aerogel particles.

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 4). ADO presence isn't detected at the developed formulations by FTIR.

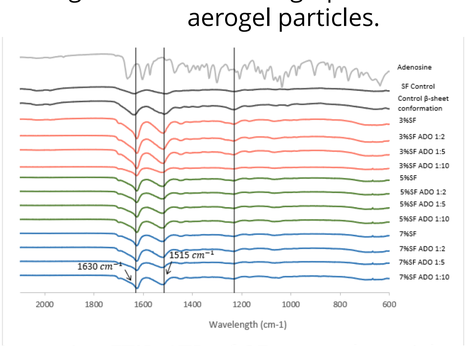

Figure 4 – FTIR-ATR of SF aerogel particles and controls .

Cell viability of SF Aerogel particles without adenosine were tested using HDF's cell line. After 7 days of incubation, cell viability was higher than in the control, thus indicating that aerogel particles promote cell proliferation (Figure 5a). These results were confirmed by SEM analysis (Figure 5b).

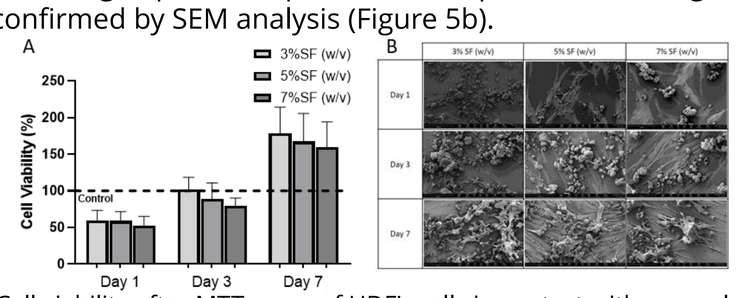

Figure 5 –A. Cell viability after MTT assay of HDF's cells in contact with aerogel particles as compared with the control group ($\alpha < 0.05$). B. SEM micrographs of HDF's cell cultures in contact with SF aerogel particles for 1, 3, and 7 days.

Conclusions

This method is suitable for the production of particles. Physicochemical characteristics, drug release and cytotoxicity activity of bioactive ADO-SF particles will be explored by Helium pycnometry and N2 adsorption-desorption, HPLC method and *In vitro* assays, respectively.

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. M.C. Montesinos, A. Desai, J.-F. Chen, H. Yee, M.A. Schwarzschild, J.S. Fink, B.N. Cronstein, Adenosine Promotes Wound Healing and Mediates Angiogenesis in Response to Tissue Injury Via Occupancy of A2A Receptors, The American Journal of Pathology. 160 (2002) 2009–2018. https://doi.org/10.1016/S0002-9440(10)61151-0.

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