

Structure and properties of silk fibroin based aerogel particles prepared by emulsion-gelation followed by supercritical CO₂ for wound healing

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Introduction

Chronic wounds are one of the major therapeutic and healthcare challenges. The wound healing process is divided in three important stages (haemostasis, inflammation and proliferation/maturation) that deploy a series of biochemical reactions which induce the repair of the injury. During wound healing, a fluid (exudate) can be produced as a natural response towards healing. However, its excessive production can be detrimental, once it is an excellent culture medium for bacterial growth, delaying the inflammatory phase. Nowadays, the design and development of biocompatible, biodegradable and adaptable materials that promote the tissue repair, prevent the infection and inflammation and ensure the management of exudate are a constant need for wound management.

Aerogels are nanostructured dry materials with high porosity, large surface and low bulk density (López-Iglesias et al., 2019). They can provide advanced performance for wound healing due to their high porosity and large surface area, which can be tailored for a fast and directional fluid transfer of the exudate. They can also act as carriers for bioactive compounds and can present an adequate biological performance to provide the required wound physiological environment. Silk fibroin (SF) protein, obtained from *Bombyx Mori*, is an excellent carrier of bioactive compounds while supporting cell proliferation, being presently used in wound healing and regeneration. In this work, we propose the use supercritical CO₂ technology to develop SF aerogel particles as a controlled release system of adenosine, a protein that is herein proposed for the first time being expected to trigger the healing process of chronic wounds, promoting angiogenesis, reducing inflammation and accelerating wound closure (Figure 1).

Experimental Methods

Silk fibroin extracted from *Bombyx mori* cocoons was used to prepare SF aerogel particles by emulsion-gelation from SF aqueous solutions at different concentrations (3%

and 5% (w/v)) as aqueous phase in water-in-oil emulsions followed by supercritical CO₂ drying (Figure 2A). The oil phase was prepared by using paraffin oil with different percentages of Span 80 surfactant. For the characterization of the SF particles, Fourier Transform Infrared with Attenuated Total Reflectance (FTIR-ATR) spectroscopy were used to investigate the secondary structure formation and conformation. The chemical structure and crystallinity of the produced matrices were analysed by FTIR-ATR and X-Ray Diffraction (XRD). Textural properties were assessed by helium pycnometry and N₂ adsorption-desorption (BET) tests. The drug loading capacity and release of adenosine in SF aerogel will be evaluated. In vitro tests will be performed using human skin cells to access the cell viability and therapeutic effects of the developed systems.

Results and Discussion

SF gel particles were produced with an emulsion-gelation method using different concentrations of SF, stirring velocity (600 and 1100 rpm) and Span 80. Namely, the SF particles with a stirring velocity of 600 rpm were more spherical and homogeneous (Figure 2B). The diameter of the particles (Dv10 and Dv90) was $509 \pm 3 \mu\text{m}$ and $1073 \pm 91 \mu\text{m}$ for 5% SF aerogel particles and $368 \pm 2 \mu\text{m}$ and $1075 \pm 22 \mu\text{m}$ for 3% SF aerogel particles, considering the average size of all the methodologies adopted.

The obtained aerogels were characterized in terms of textural and chemical properties. Analysis of the surface area of 5%SF (w/v) and 3%SF (w/v) by BET. All aerogels had high surface area being the 5%SF solution higher than 3%SF solution at the same conditions, 3%Span80 and 600rpm ($515 \pm 26 \text{ m}^2/\text{g}$ and $458 \pm 23 \text{ m}^2/\text{g}$, respectively). The surface area was lower at 5%SF and 3%SF with 3%Span80 and 1100rpm ($147 \pm 7 \text{ m}^2/\text{g}$ and $177 \pm 9 \text{ m}^2/\text{g}$, respectively). The surface area of 5%SF (w/v) particles with 10%Span and 600 rpm was $160 \pm 8 \text{ m}^2/\text{g}$. According to ATR-FTIR 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 amide I and II regions (Figure 2C). Also, it was detected the presence of Span80. The non-complete removal of Span80 may explain why the surface area of the SF aerogels with 10%Span is lower. According to XRD analysis, it is possible to verify the existence of a broad peak referring to SF (Figure 2D).

Conclusion

Physicochemical and textural characterization of the SF aerogels showed excellent properties and low particle size deviation, suggesting that the method is suitable for the production of particles for wound healing applications. In the future, we intend to load these particles with pharmaceutical drugs relevant for wound healing applications.

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