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

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

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.[1] Aerogels are nanostructured materials with high porosity, large surface area, low bulk density and water uptake that can provide advanced performance for wound healing.[1] Silk fibroin (SF) aerogels can act as promising carriers of bioactive molecules while supporting cell proliferation.[2] Hereupon, SF aerogels were developed in the form of particles for wound healing applications.

EXPERIMENTAL METHODS

Silk fibroin 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 to absolute ethanol and Span 80 (3 wt.% with respect to SF), followed by supercritical CO_2 drying (120 bar, 39ºC, 3.5 h). SF particle size distribution were characterized by laser diffraction. Fourier Transform Infrared with Attenuated Total Reflectance (FTIR-ATR) spectroscopy was used to study the chemical structure, in particular secondary structure formation. Textural properties were analyzed by helium pycnometry and N_2 adsorption-desorption. Aerogel particles biocompatibility was 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 AND DISCUSSION

SF aerogel particles were characterized concerning particle size distribution. The average diameter and the dispersion increased with increasing SF concentration. These particles presented also high surface area and low skeletal density. 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. SF aerogel particles were tested by MTT assay and the cell viability increases consistently with time. After 7 days the cell viability is considerably higher as compared to the control, suggesting that aerogel particles can promote cell proliferation. These results were confirmed by SEM analysis.

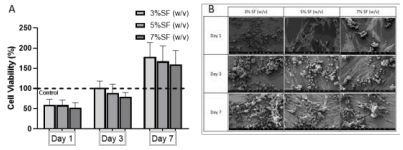

Figure 1 – A) Cell viability after MTT assay of HDF's cells in contact with aerogel particles as compared with the control group. No statistical difference was observed between groups for the same time point ($\alpha < 0.05$). B) SEM micrographs of HDF's cell cultures in contact with SF aerogel particles for 1, 3, and 7 days.

CONCLUSION

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

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