Adenosine-Loaded Silk Fibroin: A Promising Approach for Chronic Wound Healing and Regeneration

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Introduction/Resume

Chronic wounds are one of the major therapeutic and healthcare challenges affecting the population globally. During the healing stage of a wound the production of exudate is considered as a normal process. However, its overproduction can compromise and delay the inflammatory phase, contributing to wound chronicity. Aerogels are highly porous materials which can provide advanced performance for wound healing, as they can be tailored for a fast and directional fluid transfer of the exudate; also, they can have a therapeutic function, as carriers for bioactive compounds.[1] Silk fibroin (SF) protein is well known to stabilize bioactive molecules and therapeutic drugs while supporting cell proliferation, being presently used in wound healing and regeneration.

In this work, we propose the use supercritical CO_2 technology to develop SF aerogel particles as a controlled release system of adenosine (ADO). This nucleoside is herein proposed for the first time, being expected to trigger the healing and regeneration of chronic wounds.[2]

Methods

Development of aerogel particles

SF aqueous solutions at different concentrations (3, 5 and 7% (w/v)) loaded with ADO at different weight ratios (10:1, 5:1, 2:1), were introduced into absolute ethanol and Span 80 followed by supercritical CO_2 drying (Figure 1). Scanning Electron Microscopy (SEM) was used to analyze the morphology of the particles and to visualize their interaction with; laser diffraction was performed to determine particles diameter. The biocompatibility was assessed using three types of cells that play an important role during wound healing, human dermal fibroblasts (HDF), human immortalized keratinocytes (HaCaT) and human dermal microvascular endothelial cells (HDMEC). The interaction between ADO-loaded SF aerogel particles was assessed by viability and proliferation assays. Quantitative data were subjected to an analysis of variance (one-way ANOVA, Tukey's test; α=0.05).

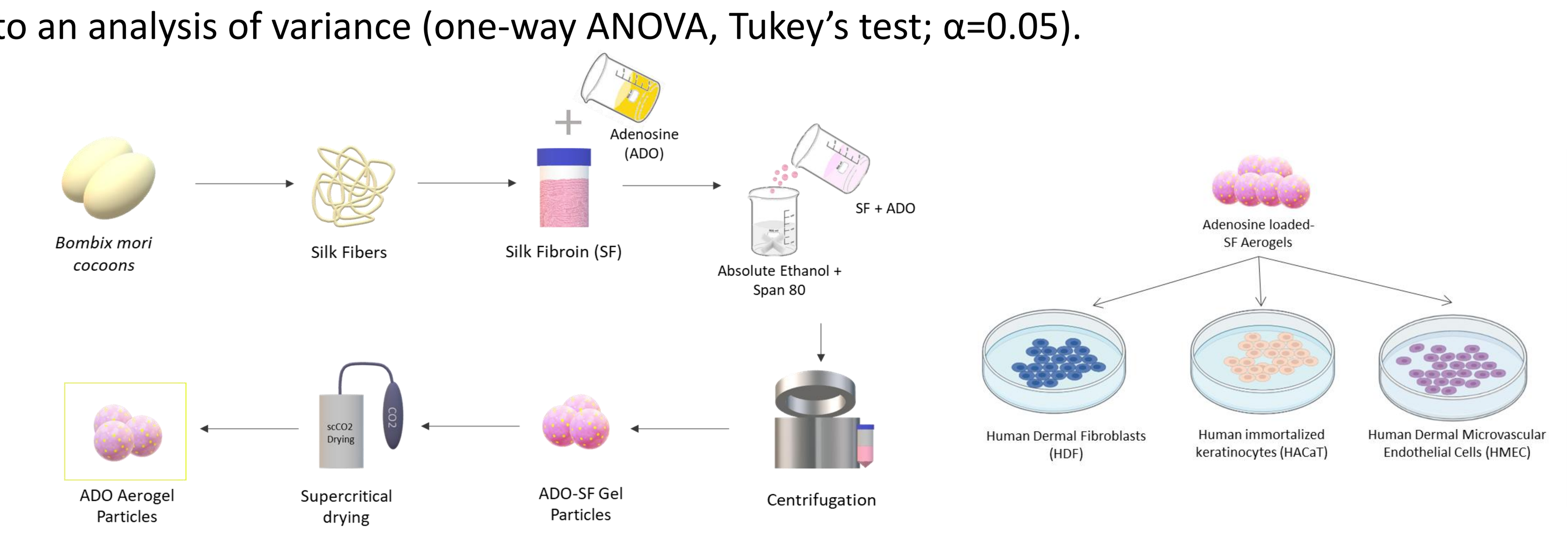


Figure 1. Silk-based aerogel particles production method (left) and *In vitro* tests (right).

Results

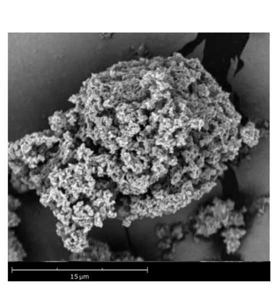
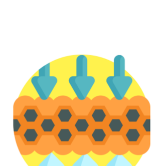
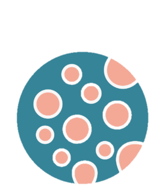


Figure 2. SEM micrographs of Silk-based aerogel particles.

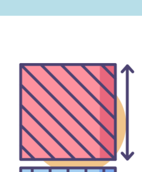
Average Particles Diameter (Dv50)
18.00 ± 3.48 μm



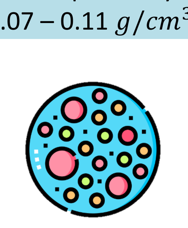
Porosity: 93-94%



Envelop Density:
$0.07 - 0.11\ g/cm^3$

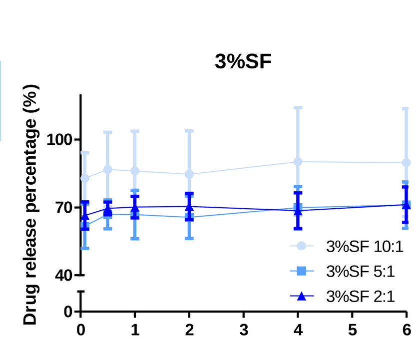


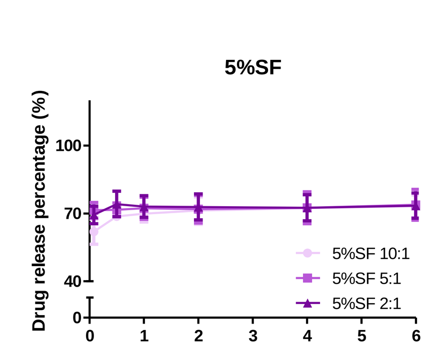
Surface Area:
$193 - 206\ m^2/g$

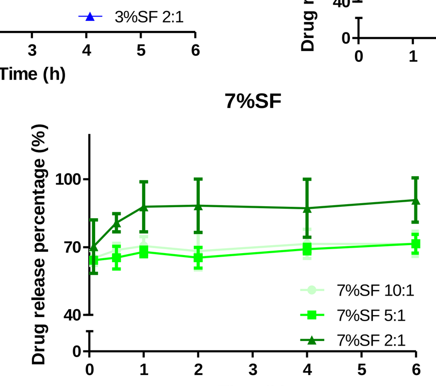


Skeletal Density:
$1.25 - 1.31\ g/cm^3$

Drug release (%)







Cell viability

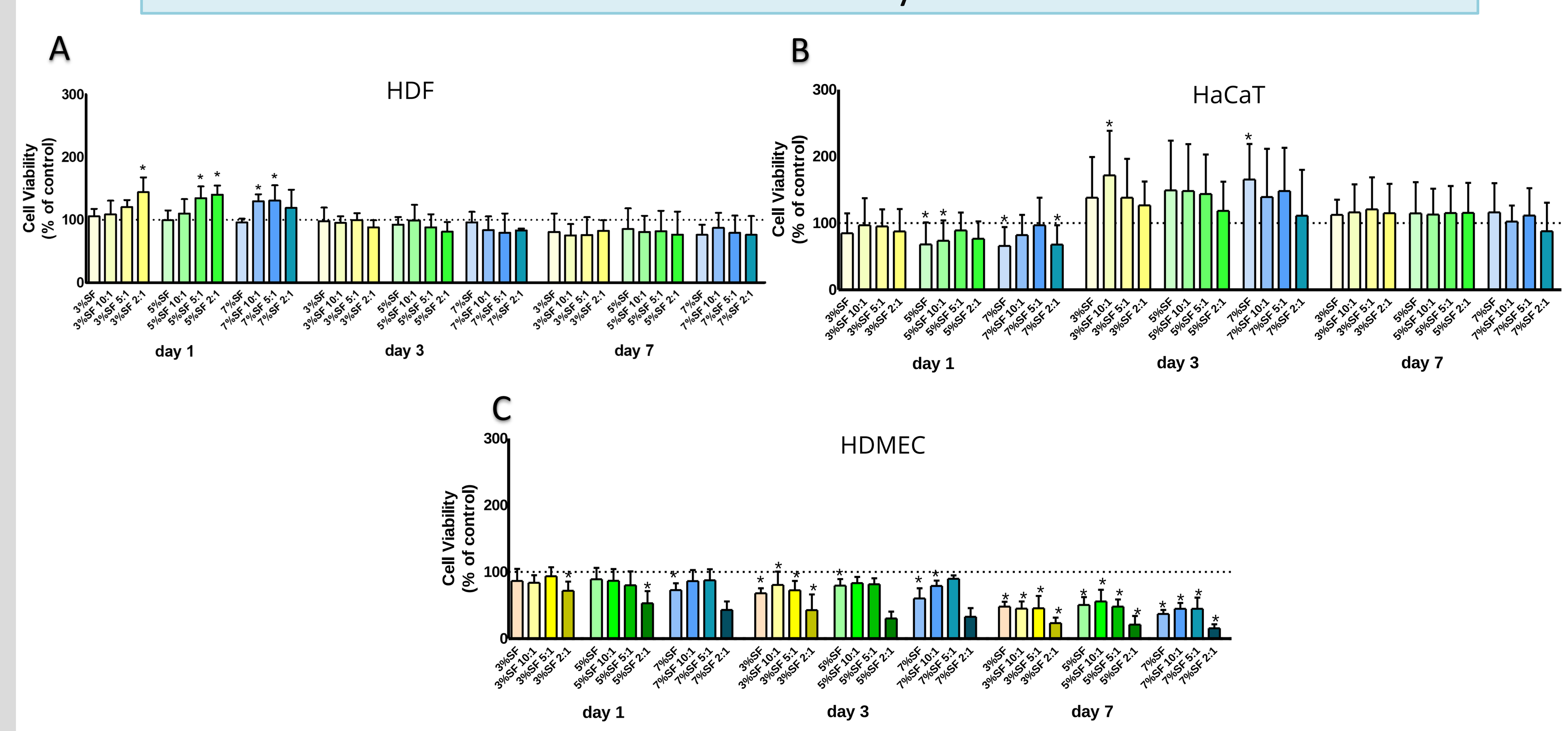


Figure 4. Cell viability after Resazurin assay of HDF (A), HaCaT (B) and HDMEC (C) cells in contact with aerogel particles as compared with the control group (α < 0.05).

Cell proliferation

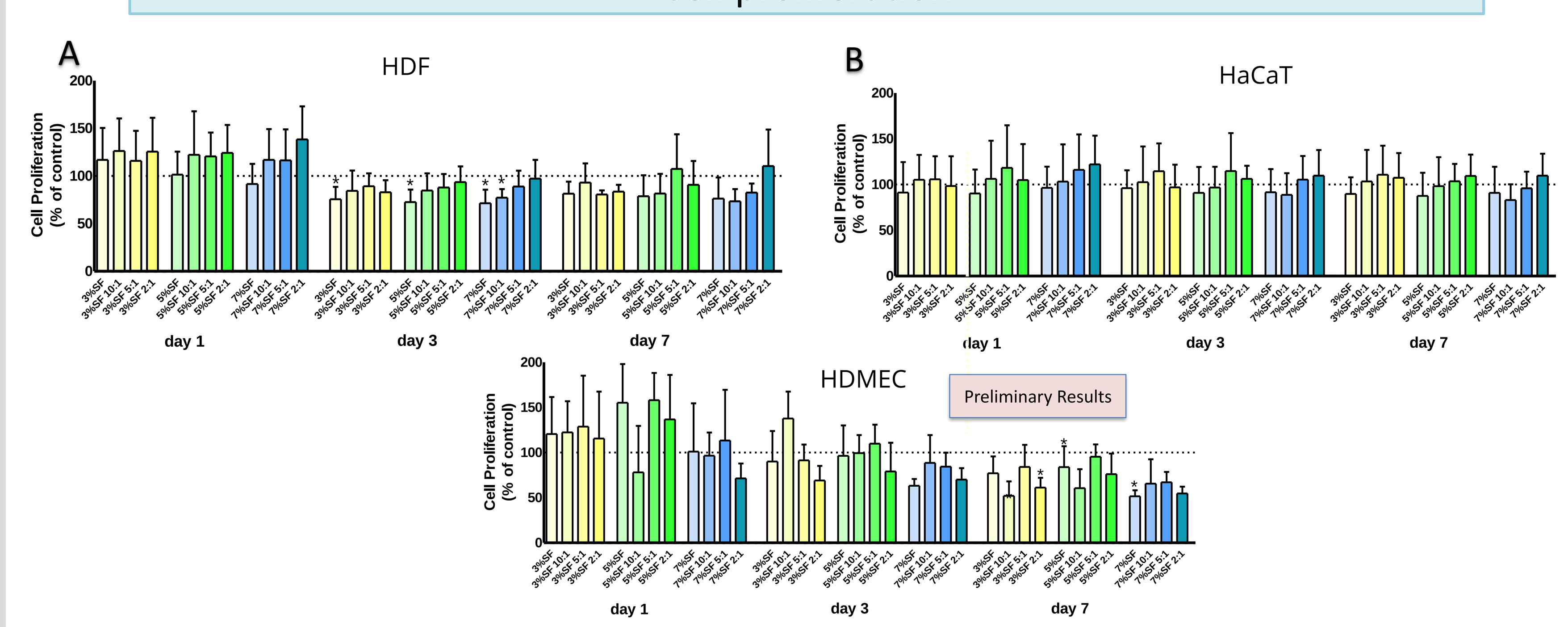


Figure 5. Cell proliferation BrdU of HDF (A), HaCaT (B) and HDMEC (C) cells in contact with ADO-aerogel particles as compared with the control group (α < 0.05).

SEM

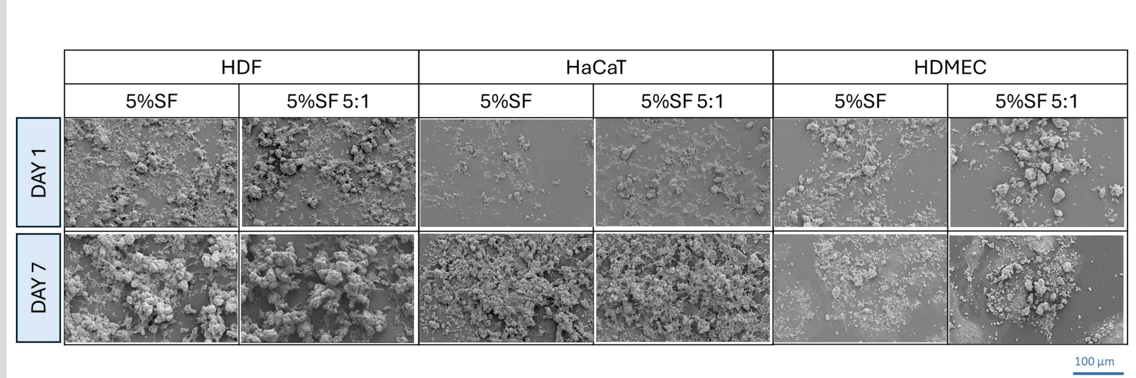


Figure 6. SEM micrographs of HDF, HaCaT and HDMEC cell cultures in contact with SF aerogel particles.

Immunofluorescence

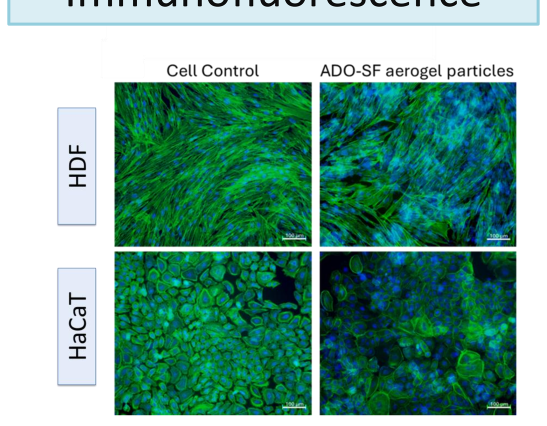


Figure 7. Representative immunofluorescence images of HDF and HaCaT cells treated with SF aerogel particles.

Conclusions

The microparticles showed favorable morphological properties and supported cell proliferation and biocompatibility, with drug release tests indicating rapid adenosine release. Ongoing assays with HDMEC indicate favorable cell behavior, providing insights into their angiogenic properties. Future work involves optimizing formulations for enhanced therapeutic efficacy and exploring clinical applications in chronic wound management and tissue regeneration.

Bibliography

1. B. G. Bernardes, P. del Gaudio, P. Alves, R. Costa, C. A. García-Gonzaléz and A. L. Oliveira, Molecules, 2021, 26, 3834.
2. M. C. Montesinos, A. Desai, J.-F. Chen, H. Yee, M. A. Schwarzschild, J. S. Fink and B. N. Cronstein, Am J Pathol, 2002, 160, 2009–2018.

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