Unleashing the Healing Potential of Adenosine: Advanced Silk Fibroin Aerogel for Wound Recovery

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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

Particles' development

For the aerogel particles' production, SF aqueous solutions at different concentrations (3, 5 and 7% (w/v)) loaded with ADO at different ratios (2:1, 5:1, 10:1) were introduced into an absolute ethanol and Span 80 followed by supercritical CO2 drying (Figure 1). Scanning electron Microscopy (SEM) was used to analyze the morphology of the particles and to visualize the interaction of cells with them; laser diffraction was performed to determine particles diameter. The biocompatibility was assessed using two types of cells that play an important role during wound healing, human dermal fibroblasts (HDF) and human immortalized keratinocytes (HaCaT). The interaction between ADO and 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.

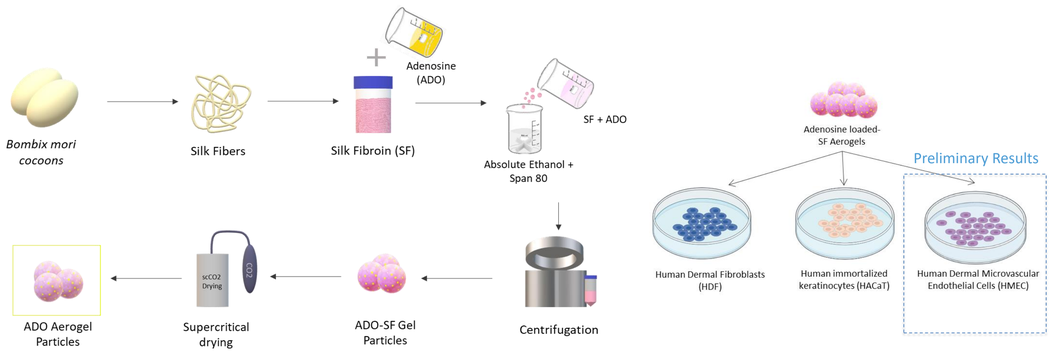

Figure 1. Silk-based aerogel particles production method and *In vitro* tests.

Results

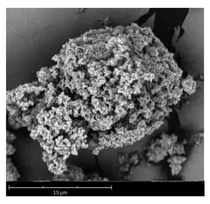

Figure 2. SEM micrographs of Silk-based aerogel particles.

Particles	Dv50 (μm)	ε (%)
3%SF	19,4 ± 0,03	93 ± 0.02
3%SF 10:1 (SF:ADO)	23,5 ± 0,53	93 ± 0.02
3%SF 5:1 (SF:ADO)	16,2 ± 0,04	93 ± 0.04
3%SF 2:1 (SF:ADO)	15,1 ± 0,08	94 ± 0.01
5%SF	22,4 ± 0,06	92 ± 0.02
5%SF 10:1 (SF:ADO)	12,1 ± 0,04	92 ± 0.01
5%SF 5:1 (SF:ADO)	17,2 ± 0,02	92 ± 0.03
5%SF 2:1 (SF:ADO)	17,8 ± 0,04	93 ± 0.01
7%SF	17,4 ± 0,04	92 ± 0.02
7%SF 10:1 (SF:ADO)	14,2 ± 0,05	93 ± 0.02
7%SF 5:1 (SF:ADO)	17,2 ± 0,08	92 ± 0.03
7%SF 2:1 (SF:ADO)	22,4 ± 0,26	94 ± 0.03

Table 1. Characterization of ADO-SF Aerogel particles. Notation: Dv50, maximum particle diameter below which 50% of the sample volume exists; ε, porosity.

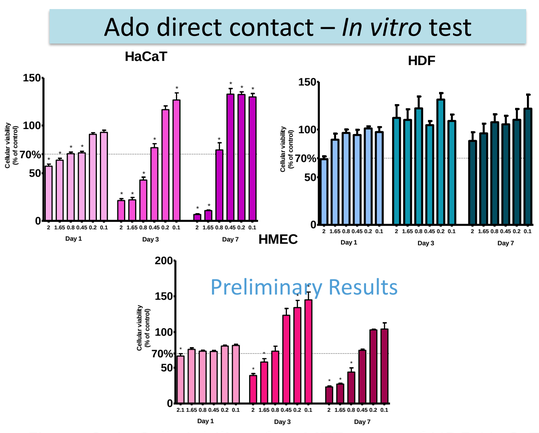

Figure 3. A. Cell viability after MTT assay of HDF, HaCaT and HMEC cells in contact with Adenosine as compared with the control group.

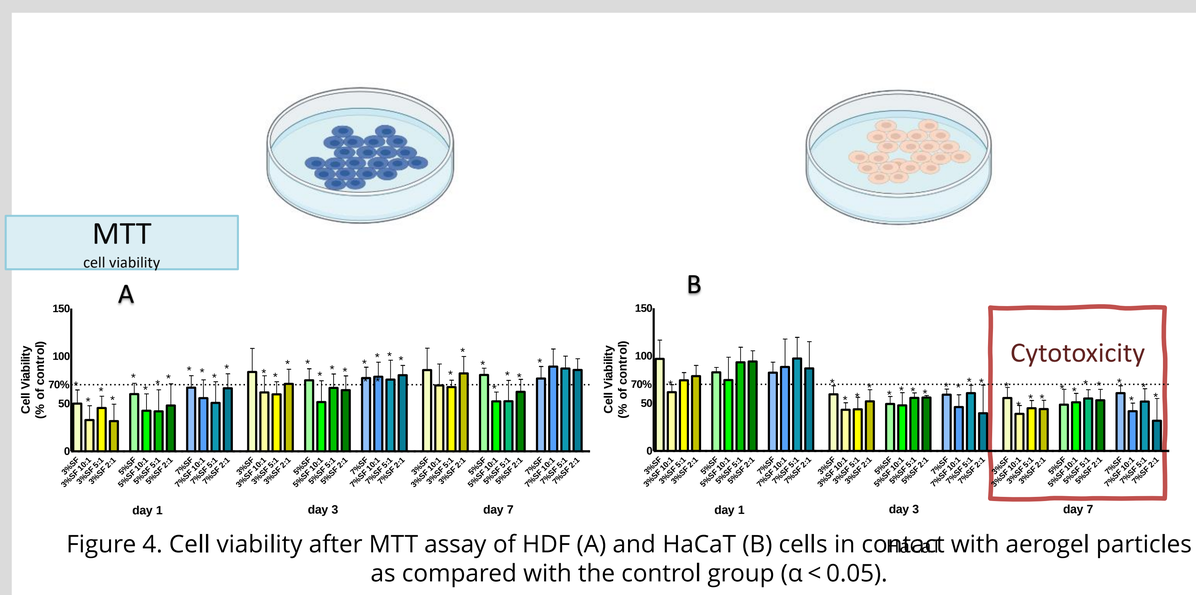

Figure 4. Cell viability after MTT assay of HDF (A) and HaCaT (B) cells in contact with aerogel particles as compared with the control group (α < 0.05).

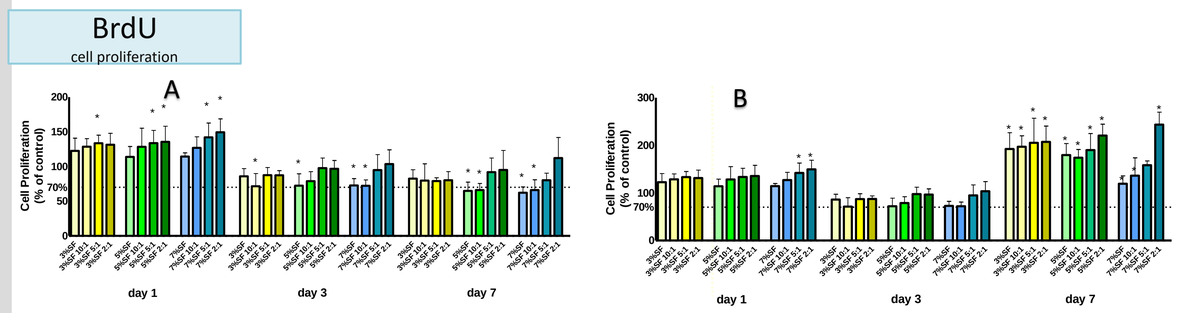

Figure 5. Cell proliferation BrdU of HDF (A) and HaCaT (B) cells in contact with ADO-aerogel particles as compared with the control group (α < 0.05).

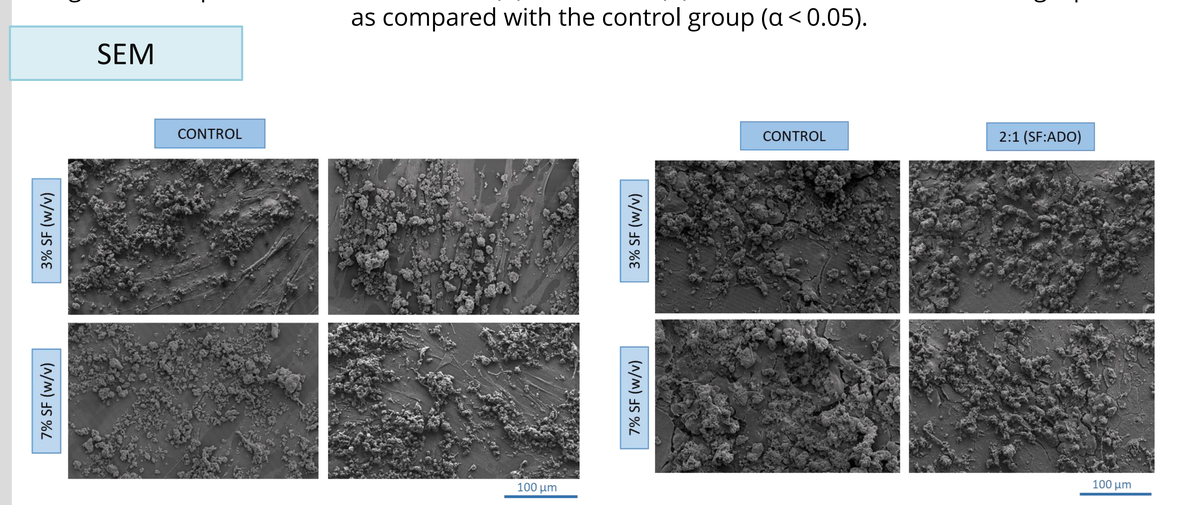

Figure 6. SEM micrographs of HDF and HaCaT cell cultures in contact with SF aerogel particles for day 3.

Conclusions

Direct contact *in vitro* tests suggests that the decrease of adenosine concentration enhances the cellular growth of HDF and the metabolic activity of HaCaT, however, the highest concentration promotes HaCaT death. The developed particles might have the potential to treat both deep and low-thickness wounds, depending on the dosage of ADO incorporated in the particles. Utilizing SEM analysis, the investigation of particle interaction with cells revealed a remarkably positive and beneficial synergy, highlighting an exceptional level of interaction. Further tests are presently ongoing to understand the therapeutic effect on endothelial cells.

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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