Abstract #480 | Oral

# Bioinspired Silk Microparticles Loaded with Adenosine: Advanced Therapeutics for Targeting Chronic Wound Healing

**B. G. Bernardes**[1, 2], R. Laurano[3], R. Costa[1, 4], A. L. Oliveira[1], C. A. García-Gonzaléz[2]

*[1] Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal*
*[2] AerogelsLab, 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, Santiago de Compostela, Spain*
*[3] Department of Mechanical and Aerospace Engineering di Torino, Politecnico di Torino, Turin, Italy*
*[4] Department of Biomedicine, Biochemistry Unit~, Faculdade de Medicina, Universidade do Porto, Porto, Portugal*

Chronic wound healing is a complex process often associated with excessive exudate production, which can prolong inflammation and raise infection risk due to polymicrobial microflora. Current wound dressings often lack efficient absorption kinetics, leading to tissue maceration and discomfort, while patient pain and inflammation management persist [1]. Aerogels, characterized by high porosity and large surface area, offer promising solutions for wound care. Bio-based aerogels, particularly those derived from natural polymers, show potential in facilitating fluid transfer and acting as carriers for bioactive compounds, thereby aiding inflammation control and infection prevention. Silk-based biomaterials have emerged as promising candidates for drug and cell delivery due to their biocompatibility, low toxicity, and customizable drug-release capabilities [2,3].

In this work we have developed new silk fibroin (SF) microparticulate aerogels loaded with Adenosine (ADO) using supercritical fluid technology, to create a drug carrier system targeting chronic wound healing to address the inflammatory stage and angiogenic properties. ADO is a nucleoside known for its ability to promote angiogenesis and tissue regeneration [4]. Various concentrations of SF (3%, 5%, and 7% w/v) and different ratios of ADO were employed for particle production. The resultant particles exhibited favorable characteristics, including high porosity (93% to 94%), an envelope density spanning 0.07 to 0.11 g/cm$^3$, skeletal density falling between 1.22 and 1.31 g/cm$^3$, and a surface area ranging from 191 to 306 m$^2$/g. The drug release tests revealed that approximately 80% of adenosine was released within 30 minutes.

*In vitro* tests were conducted using keratinocytes (HaCaT), primary fibroblasts (HDF), and endothelial (HDMEC) cells, all of which are critical to the wound healing and regeneration process. These cells contribute to the deposition of new matrix, blood vessels, and skin tissue repair. Cell viability and proliferation assays demonstrated the biocompatibility of all particle formulations with HDF and HaCaT cells (Figure 1). However, some differences were observed in the cellular interactions. Specifically, HDF exhibited favorable interaction with the particles on day 1, whereas HaCaT cells demonstrated a range of different behaviors. This variance may be linked to the superior performance of HDF over HaCaT cells when exposed to adenosine solutions within the concentration range of 0.1 – 2 mg/mL. HDMEC assays are currently underway, with preliminary findings indicating that adenosine-containing particles exhibit favorable cell biocompatibility. CAM assay is presently ongoing to evaluate the blood vessel formations in a live system.

In conclusion, the microparticles showed favorable morphological properties and supported cell proliferation and biocompatibility, with drug release tests indicating rapid adenosine release. Ongoing assays with HDMEC suggest favorable cell behaviour, particularly when adenosine, in line with its ability to promote angiogenesis. Future work involves optimizing formulations for enhanced therapeutic efficacy and exploring clinical applications in chronic wound management and tissue regeneration.

**Acknowledgment**

B.G.B. acknowledges Fundação para a Ciência e a Tecnologia (FCT) for a Doctoral Research Grant 2021.05717.BD (https://doi.org/10.54499/2021.05717.BD); Work supported by IBEROS+ (0072_IBEROS_MAIS_1_E) - Instituto de Biofabricación en Red para El Envejecimiento Saludable, funded by "Interreg VI A España – Portugal (POCTEP) 2021-2027"; by MICINN [PID2020-120010RB-I00/AEI/10.13039/501100011033], Agencia Estatal de Investigación [AEI] and FEDER funds. This publication is based on work from ECO-AERoGELS Innovation Grant (ref. IG18125) supported by COST (European Cooperation in Science and Technology). National Funds from FCT, through the project UID/Multi/50016/2020.

**Affix**

**References**

[1] Bernardes, BG.; Del Gaudio, P.; Alves, P.; Costa, R.; García-Gonzaléz, CA.; Oliveira, AL. Bioaerogels: Promising Nanostructured Materials in Fluid Management, Healing and Regeneration of Wounds. Molecules 2021, 26, 3834, doi:10.3390/molecules26133834.

[2] Bernardes, BG.; Baptista-Silva, S.; Illanes-Bordomás, C.; Magalhães, R.; Dias, JR.; Alves, NMF.; Costa, R.; García-González, CA.; Oliveira, AL. Expanding the Potential of Self-Assembled Silk Fibroin as Aerogel Particles for Tissue Regeneration. Pharmaceutics 2023, 15, 2605, doi:10.3390/pharmaceutics15112605.

[3] Baptista-Silva, S.; Bernardes, BG.; Borges, S.; Rodrigues, I.; Fernandes, R.; Gomes-Guerreiro, S.; Pinto, MT.; Pintado, M.; Soares, R.; Costa, R.; et al. Exploring Silk Sericin for Diabetic Wounds: An In Situ-Forming Hydrogel to Protect against Oxidative Stress and Improve Tissue Healing and Regeneration. Biomolecules 2022, 12, 801, doi:10.3390/biom12060801.

[4] Cronstein, BN. Adenosine Receptors and Wound Healing. The Scientific World JOURNAL 2004, 4, 1–8, doi:10.1100/tsw.2004.1.

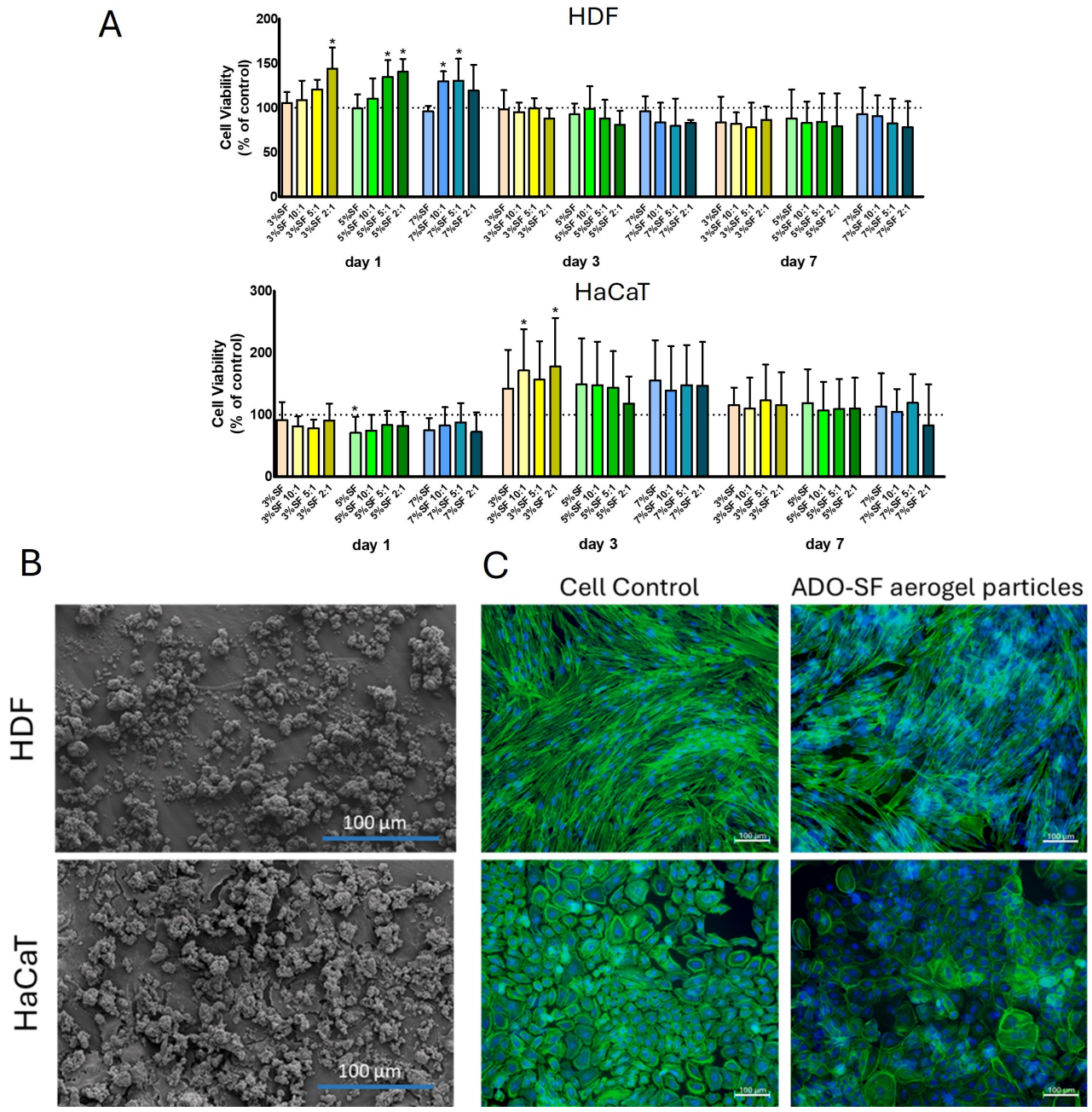


**Figure 1**

**A**. Cell viability of ADO-SF aerogel particles in contact with HDF and HaCaT; **B.** Representative SEM images of ADO-SF Aerogel particles with HDF and HaCaT; **C.** Representative immunofluorescence images showing nuclei (DAPI) and cytoskeleton (green phalloidin) of cells treated with ADO-SF Aerogel particles.