# Going green and mimetic: new ECM-based hydrogel for meniscus regeneration using supercritical $CO_2$-assisted decellularization

Chou I Ho*, Francisco A. P. Rodrigues, Mariana S. Reis, Clara Sousa, Viviana P. Ribeiro, Ana L. Oliveira, João B. Costa*

*Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia., Rua Diogo Botelho 1327, 4169-005 Porto, Portugal*
**s-chho@ucp.pt *jpbcosta@ucp.pt*

Meniscal injuries and its subsequent progression to osteoarthritis represent a major clinical challenge, as current treatments often fail to achieve effective and complete functional restoration. An alternative regenerative approach is tissue engineering using decellularized extracellular matrix (dECM), which aims to restore meniscal structure and function. Porcine meniscus, an abundant byproduct of meat production, offers a readily available source for this purpose. Unlike conventional decellularization methods that are harsh, time-consuming, and detrimental to extracellular matrix (ECM) integrity, a detergent-free decellularization protocol utilizing supercritical carbon dioxide ($scCO_2$) fluid was developed. This sustainable and minimally-invasive process was employed in cyclic pressurization-depressurization dynamics and $scCO_2$ fluid was applied for sterilization by incorporating oxidative additives.

Quantitative and qualitative analyses using quantification extraction kits and Fourier transform infrared (FTIR) spectroscopic analysis, presented as principal component analysis (PCA) and loading plots through a Machine Learning Toolbox, demonstrated substantial preservation of key ECM components. Sterilization efficiency was also confirmed using turbidity tests. dECM residual DNA content results fell within the range of general benchmark of 50ng/mg of dry tissue and the endotoxin levels, recently recognized as critical cause of host responds and regenerative outcomes [1], were below 20 endotoxin units (EU). The dECM showed cytocompatibility *in vitro* and an injectable hydrogel was successfully developed. The dECM hydrogel presented adhesive properties and was optimized to be used as a bioink for 3D bioprinting approaches.

*Acknowledgements*: Project 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.

[1] Cheng, Wenyue, *et al.* "Endotoxin, not DNA, determines the host response and tissue regeneration behavior of acellular biologic scaffolds." Acta Biomaterialia 195 (2025): 157-168.