Supercritical CO_2-Assisted Decellularization: Advanced Pancreatic Tissue Platform for Diabetes Treatment

Simone C. Sá, [a] Carlos Pazmino, [a] Joana Sá, [a] Sara Amorim, [a] Viviana P. Ribeiro, [a] Raquel Costa, [a,b,c] Ana L. Oliveira, [a]

[a] CBQF-Centro de Biotecnologia e Química Fina–Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal
[b] FMUP-Faculdade de Medicina da Universidade do Porto, Porto, Portugal
[c] i3S-Instituto de Investigação e Inovação em Saúde, Porto, Portugal

Introduction

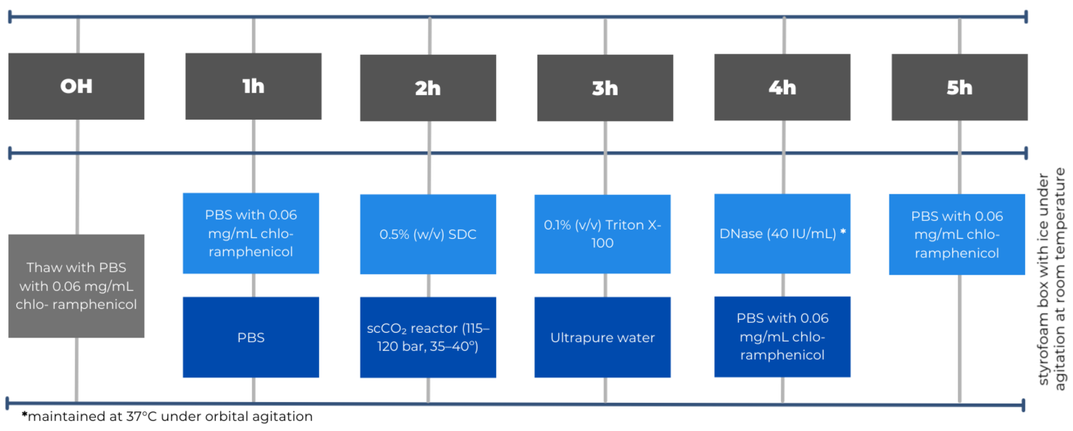
Decellularization is a procedure that aims to remove the cellular and antigenic material of a tissue while preserving its extracellular matrix (ECM) and biological properties. Decellularized ECM offers a natural microenvironment with significant potential for use in cell-based therapies and tissue regeneration [1]. Different protocols have been employed to decellularize tissues and organs. However, many rely on solvents and detergents that compromise the bioactive signals inherent to the native tissue [2]. Thus, it is crucial to establish a method that achieves effective decellularization while maintaining the ECM biochemical integrity.

Objectives

In this study, we focus on the pancreatic tissue decellularization to create a native-like matrix suitable for the delivery of functional, insulin-producing β-cells as a therapeutic approach for diabetes treatment [3].
We propose a decellularization protocol based on Supercritical CO_2 ($scCO_2$) technology as an innovative alternative to solvent-based methods, offering high transfer rates, diffusivity, chemical inertness, and non-toxicity [4].

Methods

DECELLULARIZATION PROTOCOLS



DECELLULARIZATION VALIDATION

- DNA quantification: < 50 ng of dsDNA per mg of dry sample

MATRIX CHARACTERIZATION

- Glycosaminoglycans (GAG's);
- Soluble and insoluble collagen;
- Scanning Electron Microscopy (SEM);
- Proteomic Analysis.

CITOCOMPATIBILITY EVALUATION

T0158 - Immortalized Human Pancreatic Cells – Myc

- Alamar Blue;
- BRDU

Results & Discussion

MACROSCOPIC ANALYSIS

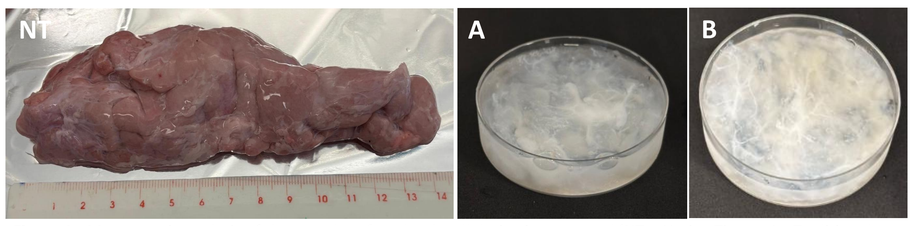
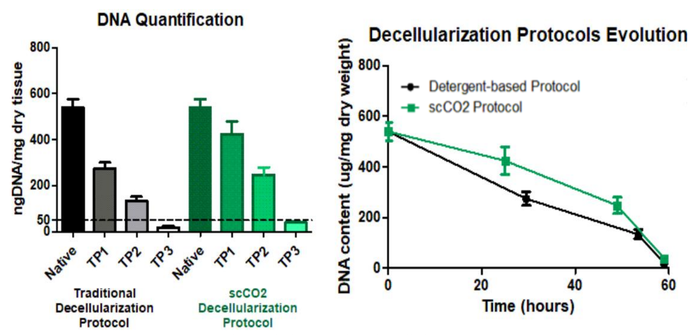


Figure 1 - Macroscopic analysis of the samples after the decellularization protocol (NT – Native Tissue; A- Traditional protocol; B- $scCO_2$ protocol).

DECELLULARIZATION VALIDATION



Decellularization rate depends on the protocol.

Figure 2 – DNA content evaluated during the decellularization protocols. Samples were collected between cycles to follow the reduction of DNA content.

MATRIX CHARACTERIZATION

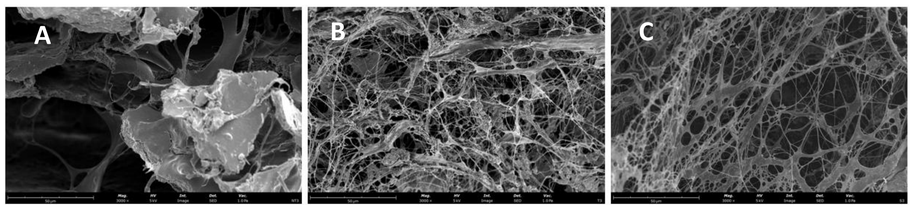


Figure 3 – (A) Native tissue, (B) traditional-decellularized, and (C) $scCO_2$-decellularized tissues. Images at 3000× magnification show ECM structure and preservation.

SEM analysis revealed increased porosity in both decellularized matrices versus native tissue.

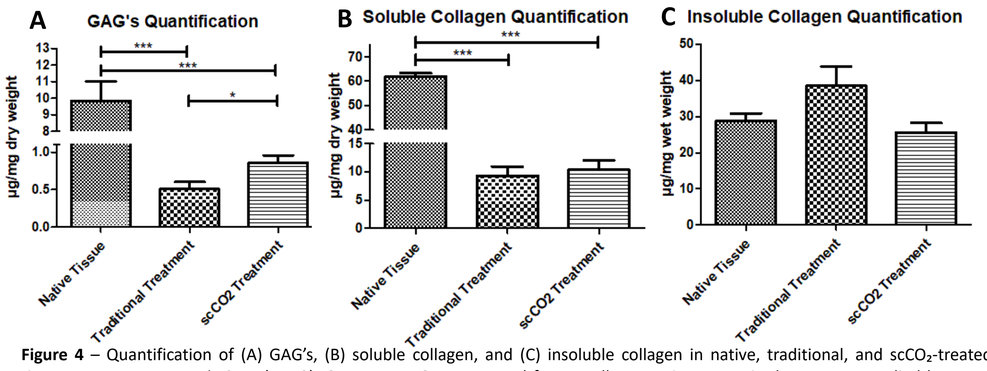


Figure 4 – Quantification of (A) GAG's, (B) soluble collagen, and (C) insoluble collagen in native, traditional, and $scCO_2$-treated tissues. Data are mean ± SEM (n = 3). One-way ANOVA was used for overall comparison; unpaired t-test was applied between decellularized groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Preservation of GAG's is slightly improved after $scCO_2$ decellularization. Insoluble collagen appears to be protocol-independent.

Traditional protocol retains higher levels of proteoglycans and SLRP's, whereas $scCO_2$ protocol better preserves some basal membrane proteins (LAMB1 and AGRN), glycoproteins, and especially elastic fiber–associated proteins, enhancing recellularization potential.

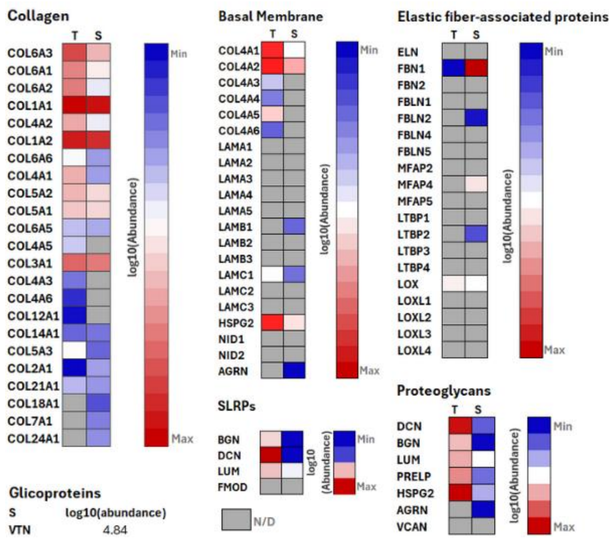


Figure 5 – ECM Proteomic analysis after traditional (T) and $scCO_2$ (S) decellularization protocols.

BIOCOMPATIBILITY

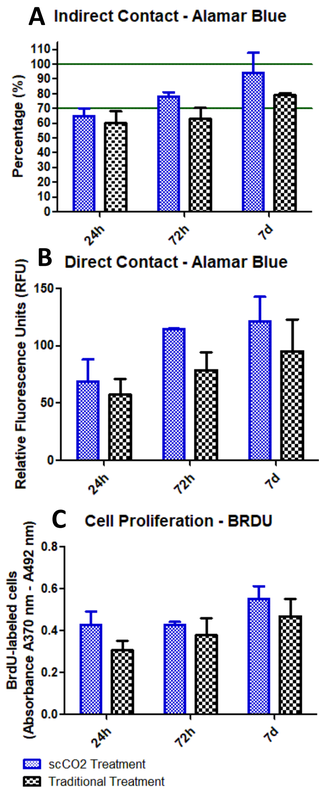


Figure 6 – Cell viability (A &B) and proliferation (C) assays at 24 and 72 hours, and 7 days. Mean ± SEM (n = 3).

Biocompatibility assays showed β-cell adhesion and proliferation on dpECM, regardless of the decellularization protocol.

Conclusions

Both decellularization protocols efficiently removed DNA and SEM confirmed ECM ultrastructure maintenance in both groups. Biochemical analysis revealed that $scCO_2$ decellularization slightly improves GAG's preservation, while insoluble collagen is protocol-independent and soluble collagen remains similar in both methods. Proteomic analysis showed that traditional treatment retained more proteoglycans and SLRPs, whereas $scCO_2$ better preserved some basal membrane and especially elastic fiber proteins. Biocompatibility assays demonstrated that both scaffolds supported cell adhesion, viability, and proliferation. In conlcusion, both protocols generate biocompatible scaffolds, with distinct ECM preservation profiles relevant for recellularization, but $scCO_2$ stands out for being a green technology, with a shorter effective decellularization time and reduced waste.

References

[1] Neishabouri, A., Soltani, A., et al. (2022). Frontiers in Bioengineering and Biotechnology. **[2]** P. M. Crapo, T. W. Gilbert, and S. F. Badylak, *Biomaterials*, vol. 32, no. 12, 2011, doi: 10.1016/j.biomaterials.2011.01.057. **[3]** Barthiya, D. (2016). Indian Journal of Medical Research. **[4]** M. M. Duarte, N. Ribeiro, I. V. Silva, J. R. Dias, N. M. Alves, and A. L. Oliveira, J. Supercrit. Fluids, vol. 172, 2021, doi: 10.1016/j.supflu.2021.105194.

Acknowledgements

Simone Costa Sá acknowledges FCT - Fundação para a Ciência e a Tecnologia for the PhD grant 2024.00955.BDANA. This work was supported by the Program Interreg VI A España – Portugal (POCTEP) 2021-2027 - project "IBEROS+ - Instituto de Biofabricación en Red para El Envejecimiento Saludable" and, by the Project "LESSisMORE: LESS disruptive technologies for advancing decellularization and postprocessing of MORE viable biological materials" financed by the Fundação para a Ciência e Tecnologia (FCT).