

Unlocking the Potential of Supercritical CO₂ for an Efficient Pancreatic Tissue Decellularization: a Step Towards an Advanced Cell Therapy

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Introduction

Tissue decellularization is a procedure that removes cellular and antigenic material from specific tissues, while preserving the extracellular matrix (ECM), with conditions tailored to the tissue's properties. The decellularized ECM is a promising material for cell-based therapies or for tissue defect repair through the development of scaffolds and membranes, among others [1]

Pancreas is an organ that regulates the glucose levels in the bloodstream, primarily through insulin secretion by endocrine pancreatic β -cells. Diabetes type 2 settles on the insufficient secretion of insulin, and the consequent increase of glucose in the body. Extensive research is being conducted to find alternatives to overcome this condition, particularly on cell-based therapies, that deliver viable and functional β -cells and restore insulin production to regulate blood glucose levels effectively [3].

Objectives

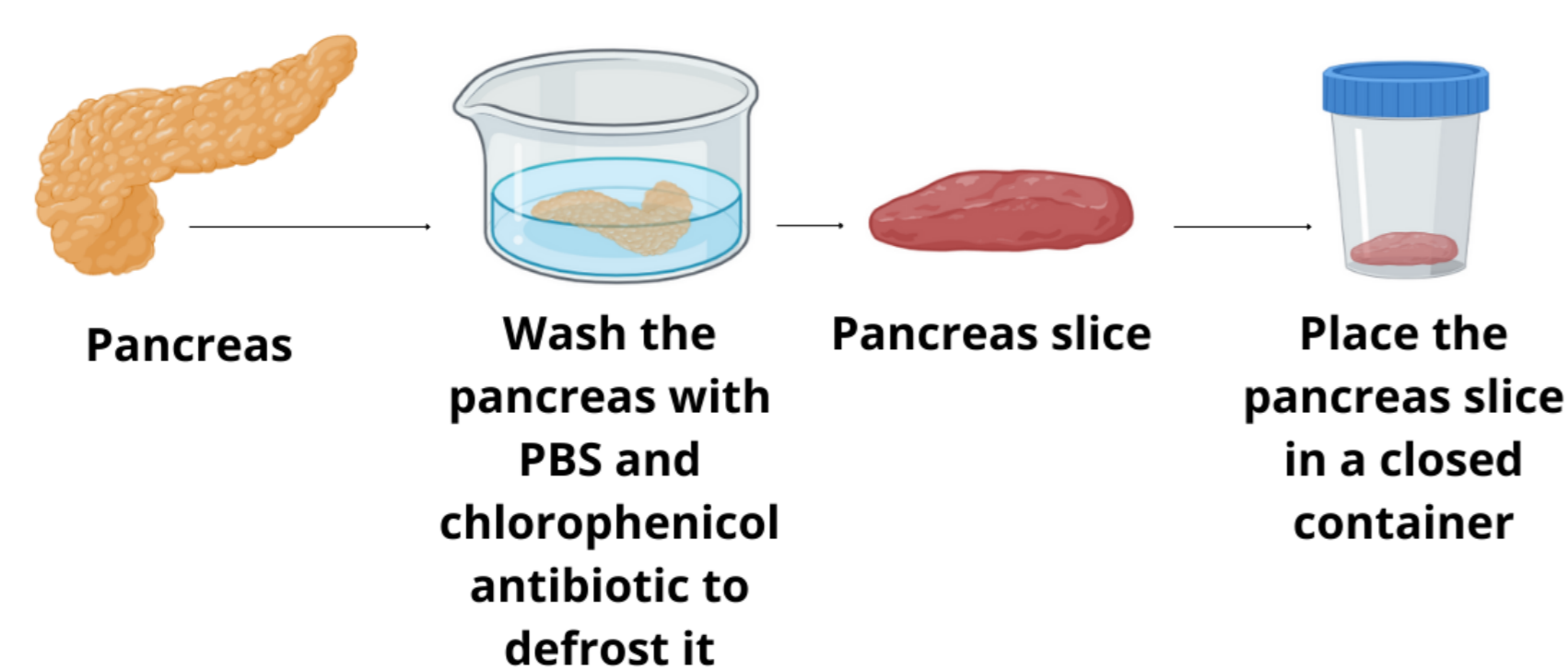
This study aims to develop and validate a decellularization protocol for porcine pancreatic tissue using Supercritical CO₂ (scCO₂) technology as an innovative alternative to solvent-based processes, offering high transfer rates, diffusivity, chemical inertness, and non-toxicity [3].

Three protocols were tested:

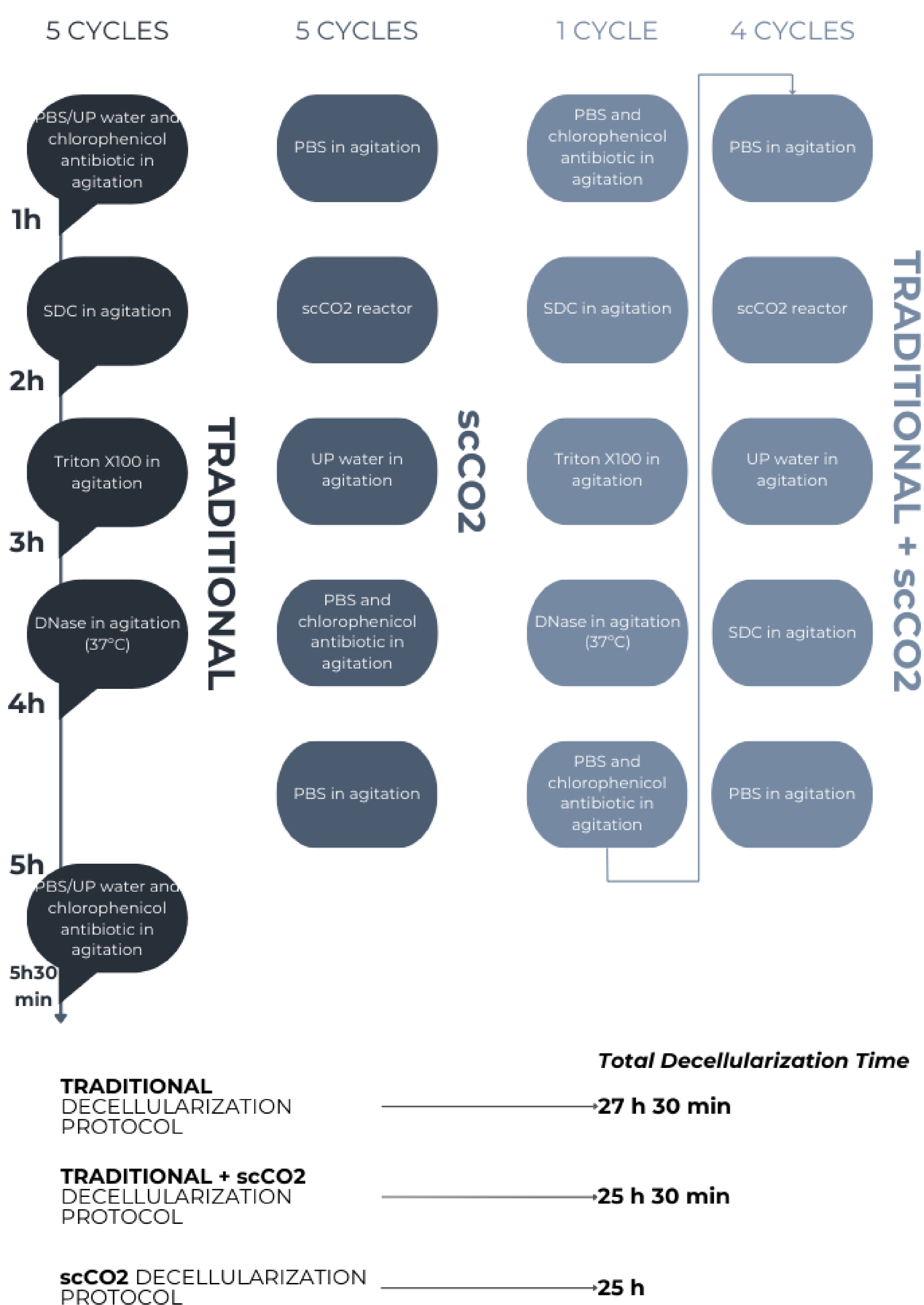
- Traditional decellularization method using detergents;
- scCO₂-based decellularization protocol;
- Hybrid protocol combining both technologies.

Materials and Methods

INITIAL PROCEDURE



DECELLULARIZATION PROTOCOLS



DECELLULARIZATION VALIDATION

- < 50 ng of dsDNA per mg of dry sample

Results and Discussion

WHAT IS THE MOST EFFICIENT TREATMENT?

Macroscopic analysis

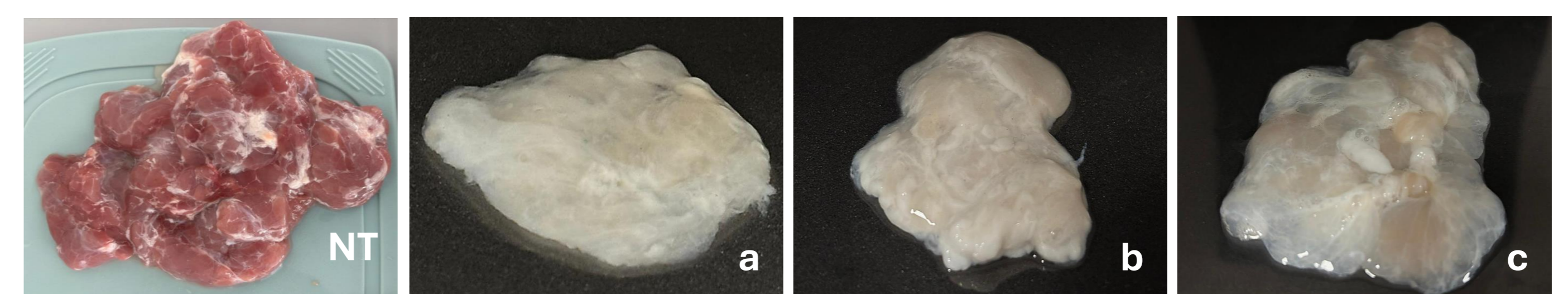


Figure 1 - Macroscopic analysis of the samples after five decellularization cycles (NT – Native Tissue; a- scCO₂ protocol; b- Traditional + scCO₂ protocol; c- Traditional protocol).

All protocols effectively reduced DNA content in porcine pancreatic tissue (Fig.1 and 2)

The scCO₂ and the traditional + scCO₂ protocols resulted in DNA concentrations below 50 ng/mL, indicating successful decellularization (Fig. 2). The scCO₂ was the fastest method, reducing DNA below 50 ng/mL in four cycles. The traditional + scCO₂ method retained more residual DNA after five cycles compared to scCO₂.

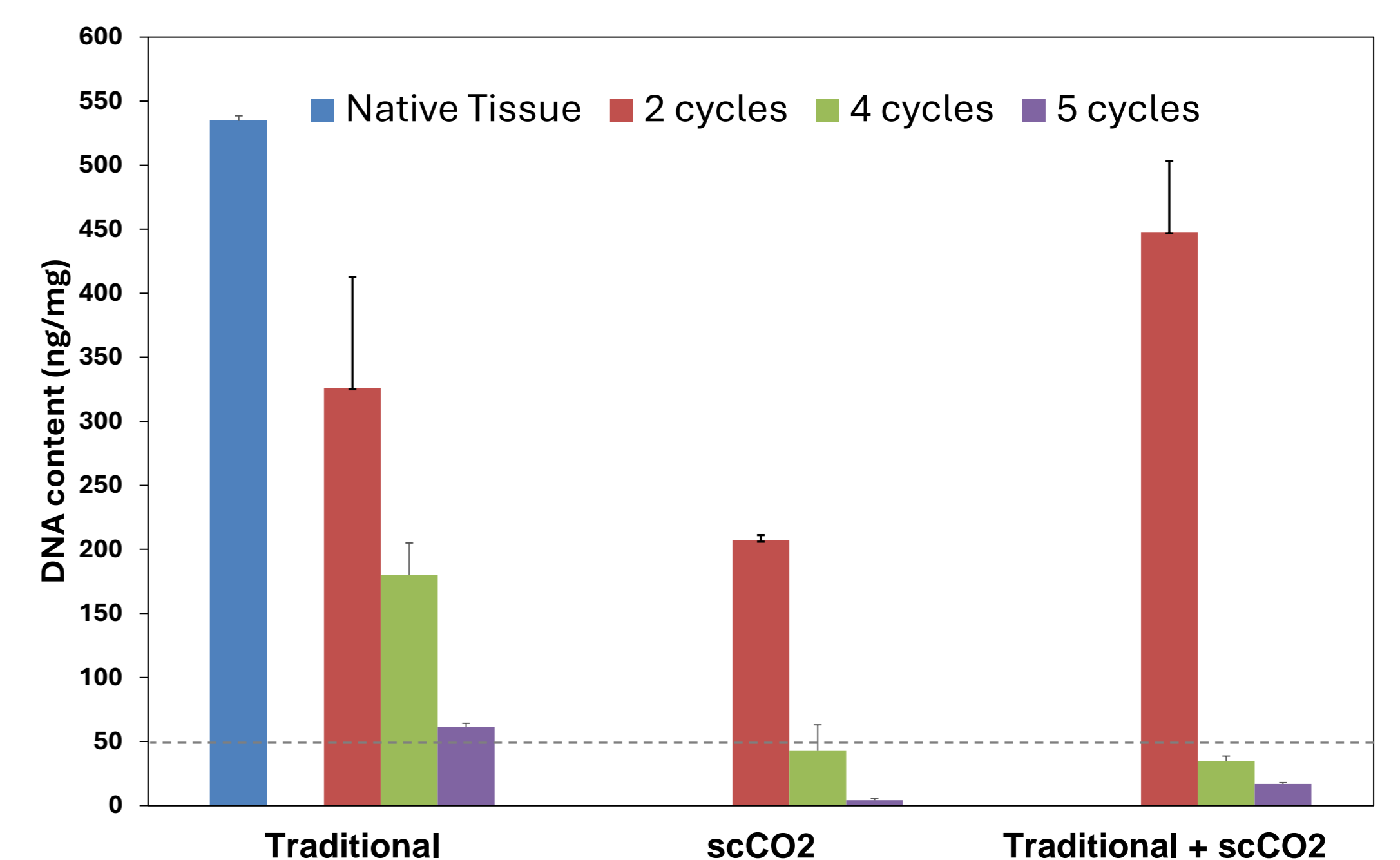


Figure 2 – DNA content evaluated during the decellularization protocols. Samples were collected between cycles to follow the reduction of cellular tissue.

Conclusions

Results suggest that scCO₂ is the most efficient method for porcine pancreatic tissue decellularization, reducing DNA below 50 ng/mL in four cycles and achieving near-complete removal by the fifth. The traditional + scCO₂ approach also meets the threshold in four cycles but retains more residual DNA than scCO₂. This highlights scCO₂ as a faster and more effective method, fully decellularizing tissue in 20 hours, while the traditional method takes 27 hours 30 minutes and remains incomplete.

References

- [1] Neishabouri, A., Soltani, A., et al. (2022). Decellularization in Tissue Engineering and Regenerative Medicine: Evaluation, Modification, and Application Methods. *Frontiers in Bioengineering and Biotechnology*. [2] Barthiya, D. (2016). Stem cells to replace or regenerate the diabetic pancreas: huge potential & existing hurdles. *Indian Journal of Medical Research*. [3] M. M. Duarte, N. Ribeiro, I. V. Silva, J. R. Dias, N. M. Alves, and A. L. Oliveira, "Fast decellularization process using supercritical carbon dioxide for trabecular bone," *J. Supercrit. Fluids*, vol. 172, no. February, p. 105194, 2021, doi: 10.1016/j.supflu.2021.105194.

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