Toward a greener approach for next-generation functional pancreatic ECM in diabetes treatment

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Introduction

Pancreas is a complex organ whose structural and functional alterations are associated with severe diseases, including diabetes [1]. Although cell-based therapies have emerged as promising strategies for pancreas regeneration, their clinical translation remains limited by the lack of biomimetic platforms that replicate the native pancreatic microenvironment [2]. Decellularized pancreatic extracellular matrix (dpECM) represents a highly promising biomimetic platform. However, efficient decellularization while preserving the structural and biochemical integrity of the ECM remains challenging. Conventional protocols rely on harsh detergents that compromise the ECM structure [3]. Supercritical carbon dioxide ($scCO_2$) has emerged as a greener and sustainable alternative for decellularization and sterilization [4].

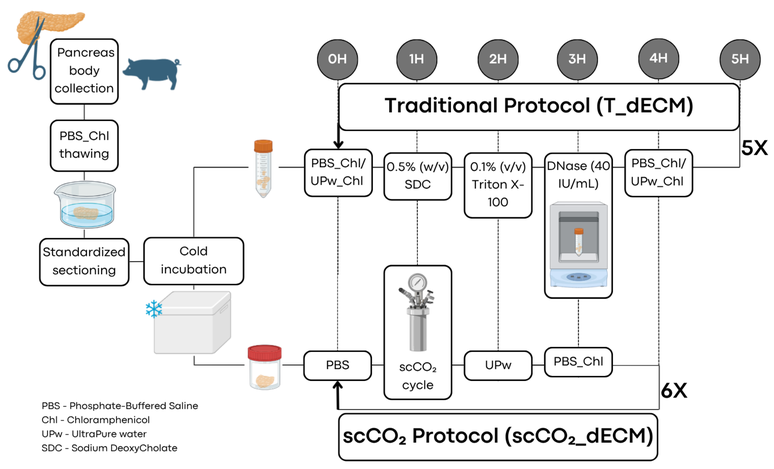
Objectives

This study aims to develop and validate a porcine pancreatic tissue decellularization protocol using $scCO_2$ technology as an innovative alternative to solvent-based methods, offering high transfer rates, diffusivity, chemical inertness, and non-toxicity [4]. To this end, two decellularization protocols were developed and tested:

- Traditional method using detergents and enzymes;
- $scCO_2$-based method.

Methods

DECELLULARIZATION METHODOLOGY



DECELLULARIZATION VALIDATION

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

MATRIX CHARACTERIZATION

- Scanning Electron Microscopy (SEM) and Transmission Electron Microscope (TEM);
- Histology Analysis (Hematoxylin and Eosin Y Alcoholic (H&E) and Masson's Trichrome (MT));
- Glycosaminoglycans (GAG's), Soluble and Insoluble Collagen Quantification;
- Proteomic Analysis.

STERILIZATION PROTOCOL ($scCO_2$ + NovaKill)

- Turbidity Assay;
- Endotoxin Quantification.

CITOCOMPATIBILITY EVALUATION

T0158 - Immortalized Human Pancreatic Cells – Myc

- Alamar Blue Assay (Cell Metabolic Activity);
- BrdU Assay (Cell Proliferation);
- Fluorescence Staining (DAPI and Green Phalloidin).

Conclusions

Both methods effectively removed cellular components and decreased DNA content by more than 90%. The traditional approach demonstrated higher efficiency in cellular debris removal, whereas $scCO_2$ better preserved ECM structure, GAGs, and tissue-specific proteins, and resulted in lower endotoxin levels. Cytotoxicity assays demonstrated that leachates from both decellularized samples are cytocompatible. Moreover, $scCO_2$ enabled effective sterilization. Overall, $scCO_2$ represents a promising sustainable alternative for pancreatic tissue decellularization and sterilization.

References

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[4] 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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Results & Discussion

INTEGRITY AND STRUCTURE OF NATIVE AND DECELLULARIZED PORCINE PANCREAS

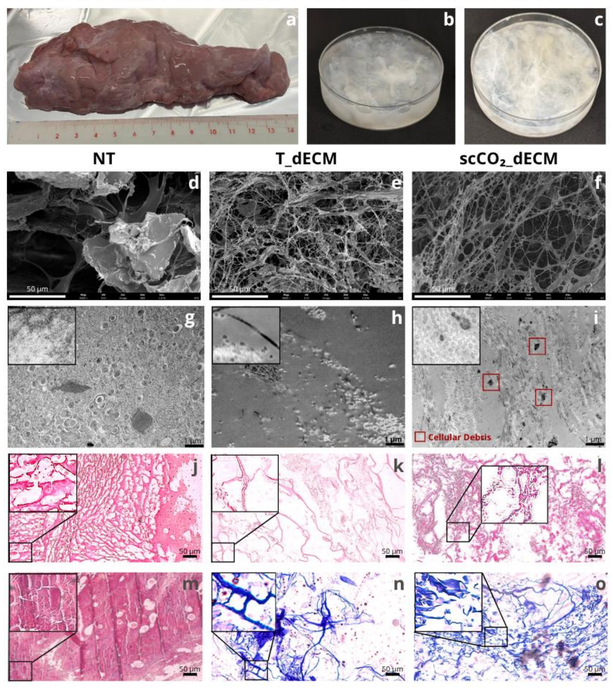


Fig. 1 *Macroscopic images of (a) NT, (b) T_dECM and (c) $scCO_2$_dECM. SEM micrographs (d-f); TEM micrographs (g-i); H&E staining of (j-l) and Masson's Trichrome staining (m-o).*

Both protocols achieved effective decellularization. The traditional method promote more efficient cell debris removal (Fig.1 g-i) resulting in less ECM structure preservation and organization compared to $scCO_2$_dECM (Fig.1 j-o).

dpECM CHARACTERIZATION

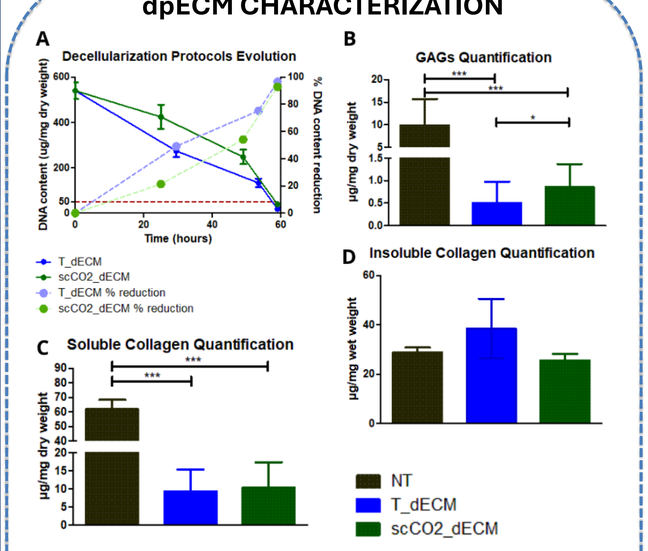


Fig. 2 *(A) DNA quantification and percentage reduction over time for traditional and $scCO_2$ decellularization protocols. (B) Sulfated glycosaminoglycans (GAGs), (C) soluble and (D) insoluble collagen quantification in NT, T_dECM and $scCO_2$_dECM.*

The $scCO_2$ protocol improved GAGs preservation. Both methods reduced soluble collagen, while insoluble collagen retention appears to be protocol-independent.

PROTEOMIC ANALYSIS

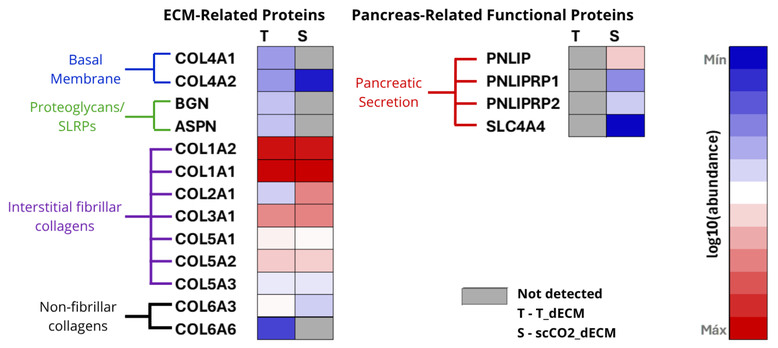


Fig. 4 *Proteomic analysis of* T_dECM and *$scCO_2$*_dECM. *Heatmaps showing the relative abundance of functional and structural ECM proteins. (S: $scCO_2$*_dECM; *T: T_dECM).*

Proteomic analysis indicates that $scCO_2$ better preserves pancreas-specific functional proteins, whereas the traditional method more effectively basal membrane proteins and proteoglycans.

CITOCOMPATIBILITY EVALUATION

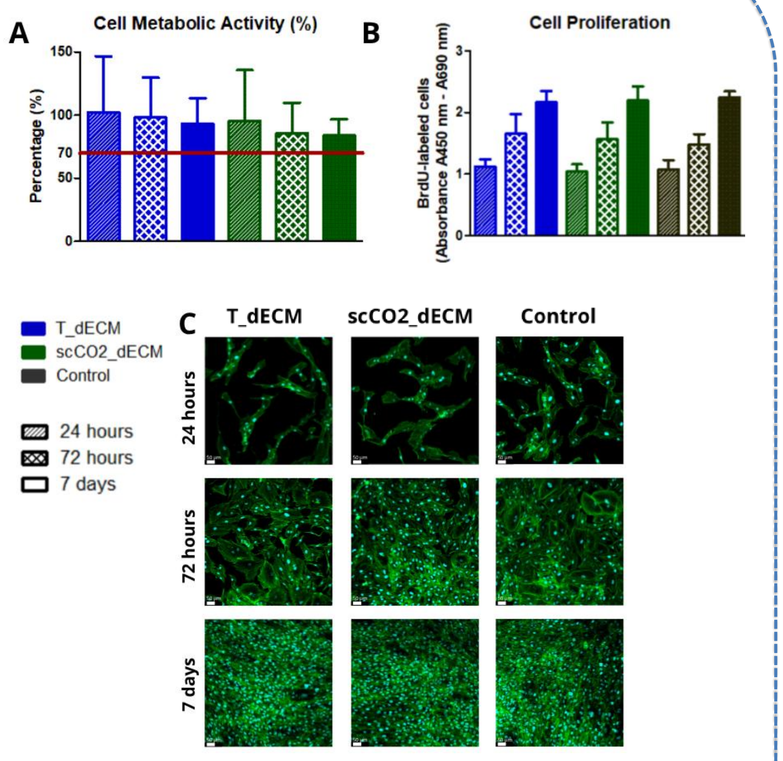


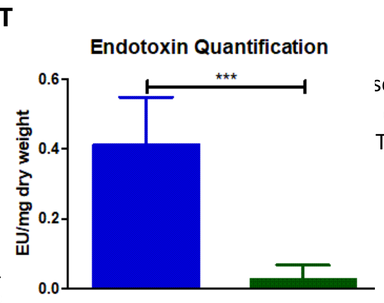
Fig. 6 *In vitro cytotoxicity and proliferation of T0158–Myc cells cultured with leachates from T_dECM and $scCO_2$_dECM samples. (A) Cell metabolic activity assessed by the Alamar Blue assay. (B) Cell proliferation assessed by the BrdU assay. (C) Representative confocal fluorescence images of T0158–Myc cells stained with DAPI (nuclei) and green phalloidin (actin cytoskeleton) after 24 hours, 72 hours, and 7 days.*

Cytotoxicity assays demonstrated that leachates from both T_dECM and $scCO_2$_dECM are cytocompatible, maintaining cell viability (>70%), supporting sustained cell proliferation, and preserving cell morphology and cytoskeletal organization. No significant differences were observed between treatments or compared to the control.

STERILIZATION VALIDATION AND ENDOTOXIN ASSESSMENT

CONDITIONS	T_dECM		scCO2_dECM	
	Inoculated & Sterilized	Inoculated & Non-Sterilized	Inoculated & Sterilized	Inoculated & Non-Sterilized
24 hours	✗	✓	✗	✓
48 hours	✗	✓	✗	✓
72 hours	✗	✓	✗	✓
7 days	✗	✓	✗	✓

The turbidity assay was performed on decellularized samples from both procedures, with both showing the same results.



Endotoxin levels were significantly lower in $scCO_2$_dECM (0.027 EU/mg dry weight) compared to T_dECM (0.413 EU/mg dry weight), highlighting the $scCO_2$ ability to remove pyrogenic residues.

Fig. 3 *(A) Turbidity assay results showing the presence (green ✓) or absence (red ✗) of bacterial growth under different experimental conditions over 7 days. (B) Endotoxin quantification of sterile decellularized pancreatic samples.*