Supercritical CO decellularized-ECM derived core-shell Hydrogel for Functional β-Cell Encapsulation and Immune Protection

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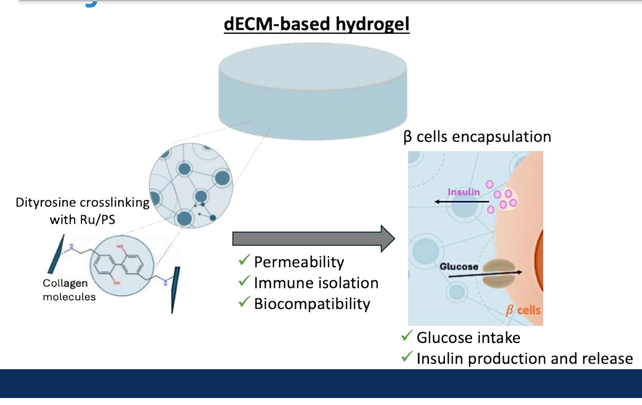
Introduction

Type 1 Diabetes Mellitus (T1DM) is an autoimmune disease that destroys insulin-producing pancreatic cells, affecting over 9 million people worldwide[1]. While insulin therapy is standard, it often leads to poor glucose control or hypoglycemia. Pancreatic islet transplantation shows promise but faces graft survival challenges. This study develops a permeable, pro-angiogenic immune-isolation hydrogel using pancreatic decellularized extracellular matrix (dECM) to mimic native tissue and support insulin production, with ruthenium as crosslinker surrounded by an Alginate shell aiming to reduce oxidative stress and inflammation. [2]

Objectives

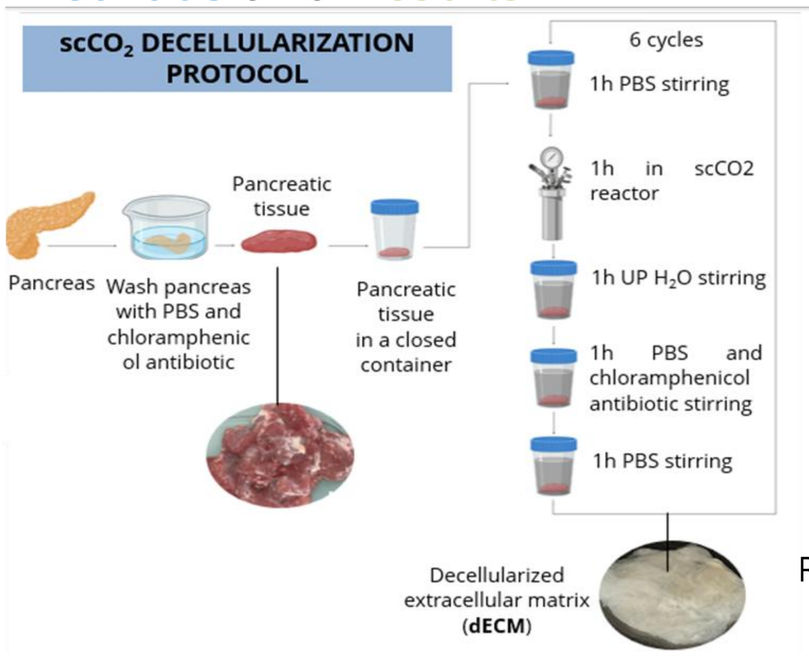


Development of a:

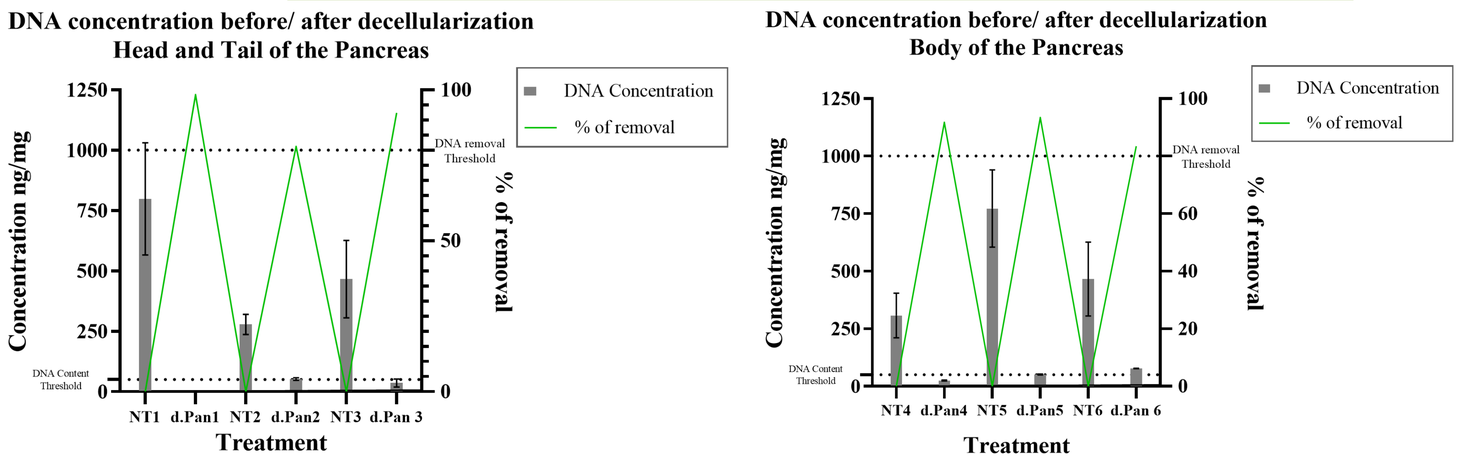
- **permeable**
- **pro-angiogenic**
- **immune-isolation**

hydrogel using decellularized pancreatic extracellular matrix (dECM) crosslinked with Ruthenium surrounded by an alginate core for the growth of beta cells.

Methods and Results



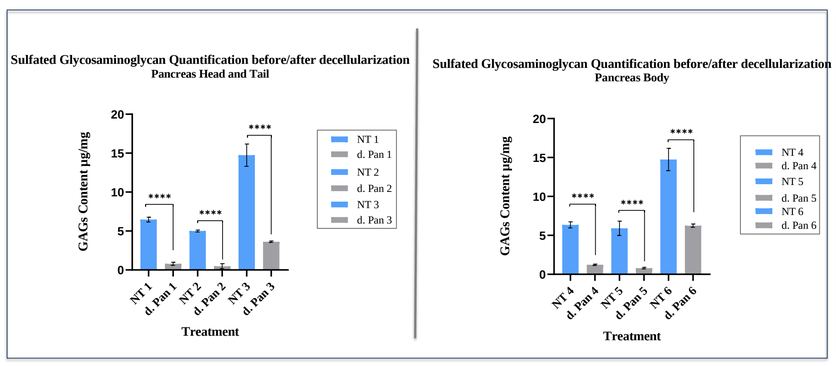
DNA Quantification



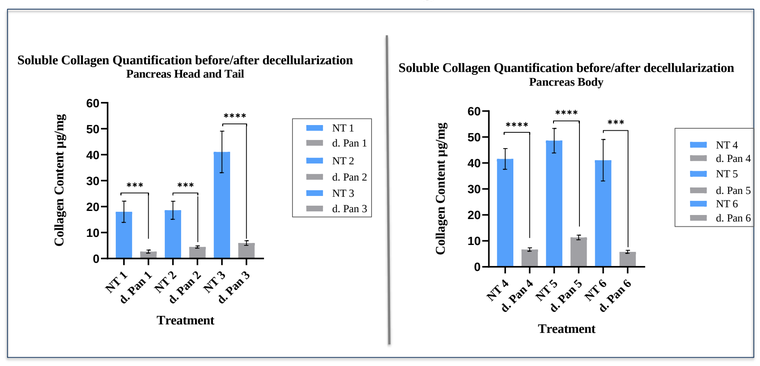
Reduced DNA content in porcine pancreatic tissue after five cycles of decellularization, resulting in a DNA concentrations **below 50 ng/mg**, indicating removal of nucleic material above 80%.

dECM CHARACTERIZATION

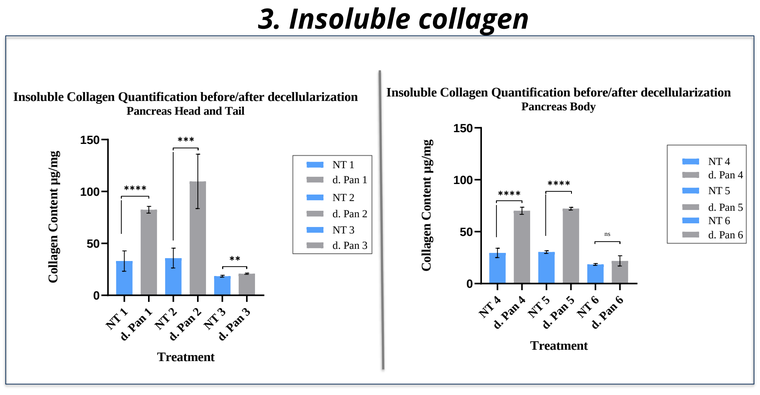
1. Gags



2. Soluble collagen

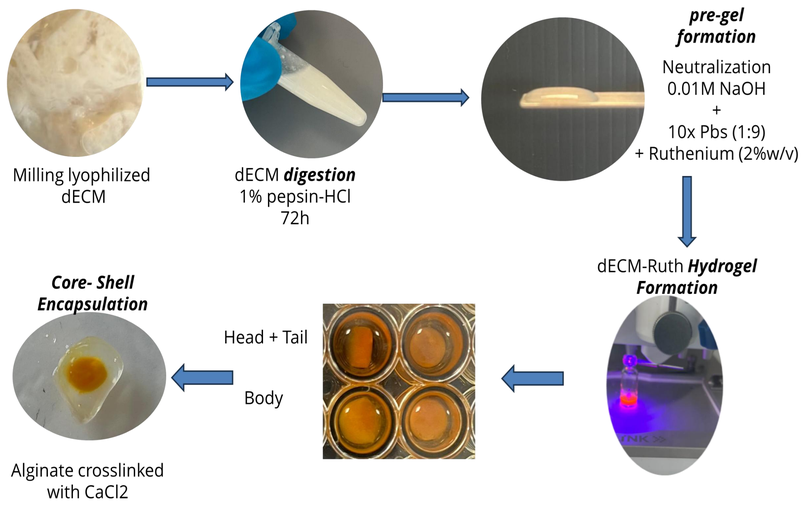


3. Insoluble collagen

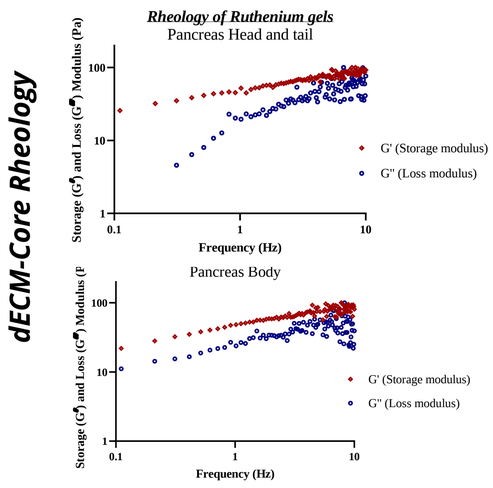


Preservation of 20% of GAGs and soluble collagen. While increased percentage of retention of insoluble collagen due to the induced crosslinking of soluble collagen during the decellularization with $scCO_2$.

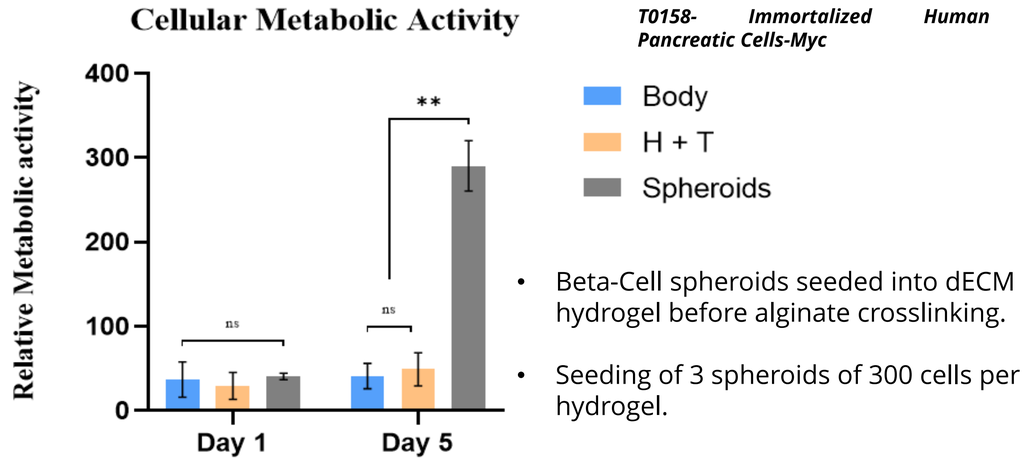
dECM DIGESTION AND CORE-SHELL HYDROGEL



- Core dECM derived hydrogel was crosslinked first followed by spheroids seeding and alginate shell crosslinking.
- Higher G″ indicates gel like characteristics for our core dECM-Ru crosslinked hydrogel.



Metabolic Activity



- Beta-Cell spheroids seeded into dECM hydrogel before alginate crosslinking.
- Seeding of 3 spheroids of 300 cells per hydrogel.
- No cytotoxicity observed.
- Anatomic areas studied present similar metabolic activities along the timeline tested.

Conclusions

This study validated a supercritical CO_2 decellularization protocol as a robust and effective method for pancreatic dECM preparation, achieving reliable DNA removal across organ variability. Decellularization reduced GAGs and soluble collagen while increasing insoluble collagen, results also highlighted regional differences within the pancreas. A core-shell hydrogel for B cell spheroids seeding was developed. The core, ruthenium-based hydrogels showed desired crosslinking but low stability after 7 days which will need to be improved. The core-shell construct showed no signs of cytotoxicity and allowed cell seeding before alginate crosslinking. Preliminary cell studies showed similar metabolic activity along the timepoints tested. Future work will focus on optimizing stiffness, reducing residual of crosslinkers in hydrogels, and expanding cellular assays. Overall, these findings establish pancreatic dECM hydrogels as promising scaffolds for β-cell replacement therapies in diabetes

References

1. Ghoneim, M.A., et al. Stem Cell Res Ther, 2024. 15(1): p. 23.
2. Alam, M.M., et al. Naseem. Arch Biochem Biophys, 2015. 584: p. 10-9

Acknowledgements

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