Detergent-free supercritical CO_2–assisted protocol for the production of sustainable and highly preserved decellularized porcine meniscus for orthopedic applications

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Introduction & Objectives

Meniscal injuries occur approximately 66 to 70 per 100,000 individuals annually (Fig. 1), potentially leading to the development of **osteoarthritis (OA)** or other degenerative cartilage disease in 10 to 20 years [1].

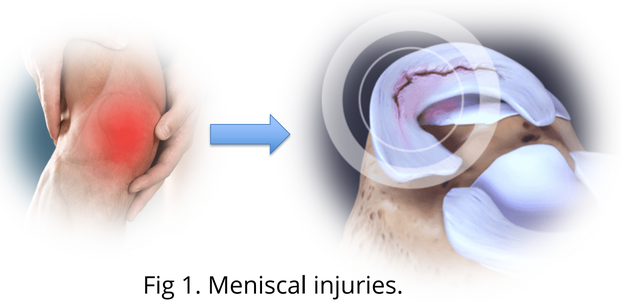


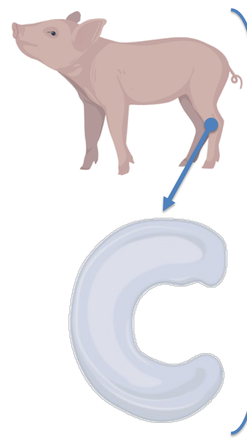
Fig 1. Meniscal injuries.

One of the conventional treatments is **meniscal allograft transplantation**. However, its limitations constrain its comprehensive application in the healthcare system [2].

LIMITATIONS

- **Concerns over longevity**
- **Limited donor availability**
- **Possible disease transmission**
- **Immune rejection-associated complications**

The development of **biomimetic meniscal scaffolds** derived from **porcine meniscus** has emerged through a **detergent-free decellularization** approach employing a **supercritical carbon dioxide ($scCO_2$)-assisted process**.



Removal of genetic material to eliminate the ability of evoking **negative immune response**

Preservation of extracellular matrix (ECM) to provide structural and biochemical signalling for the regulation of **cellular behaviours**

Methods

Two versions of $scCO_2$-assisted decellularization protocols were established, differing in the utilization of **ETHANOL** as a **co-solvent**.

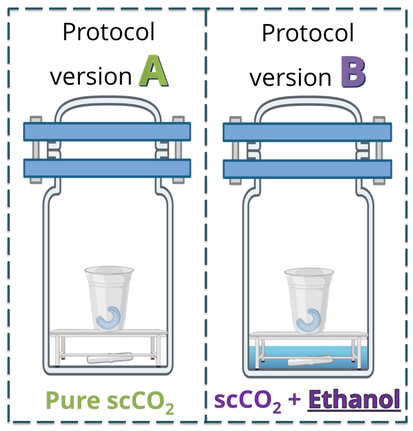


Fig 2. Diagram of meniscus in the $scCO_2$ reactor.

Cyclic protocol:

- **Sample format**: CUBE and SLICE
- **$scCO_2$ treatment cycles:** 9
- **Physical methods employed to each cycle**: freeze-thaw, direct ultrasonication, orbital shaking, and vortex
- **Solvents used**: ultrapure water, phosphate-buffered saline (PBS)
- **Protocol duration:** 5 days

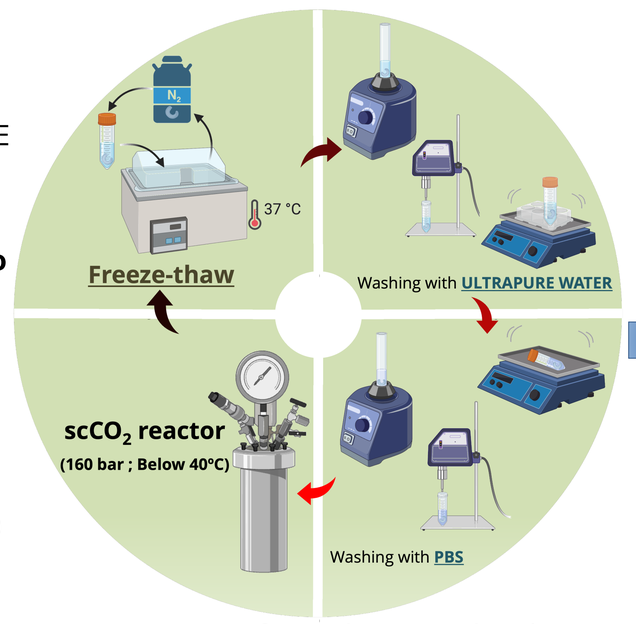
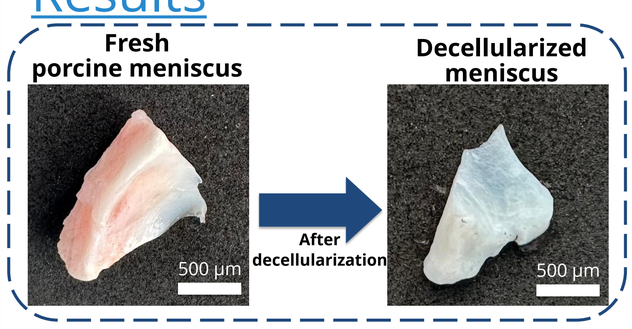


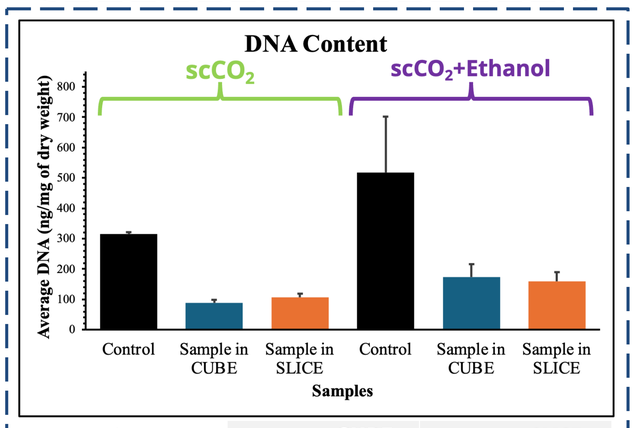
Fig 3. Diagram of procedures in each cycle.

Sterilization:

A scCO2 treatment of 3 hours was performed using a mixture of **peracetic acid** and **hydrogen peroxide** (NovaKill™) as co-solvent [3].

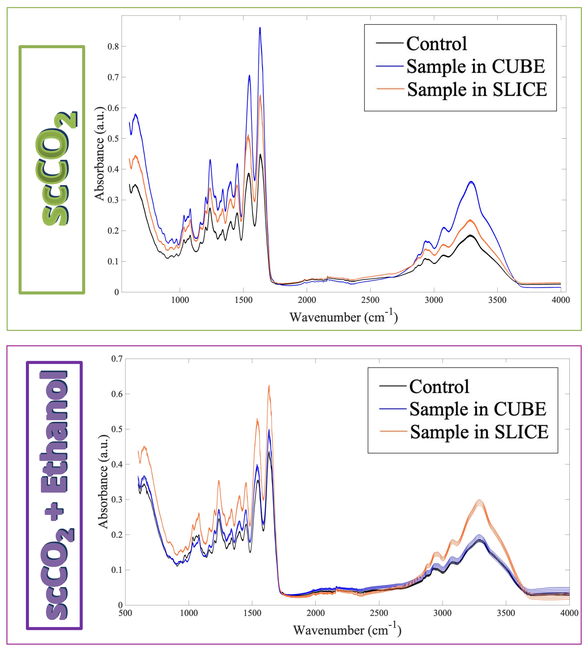
Results





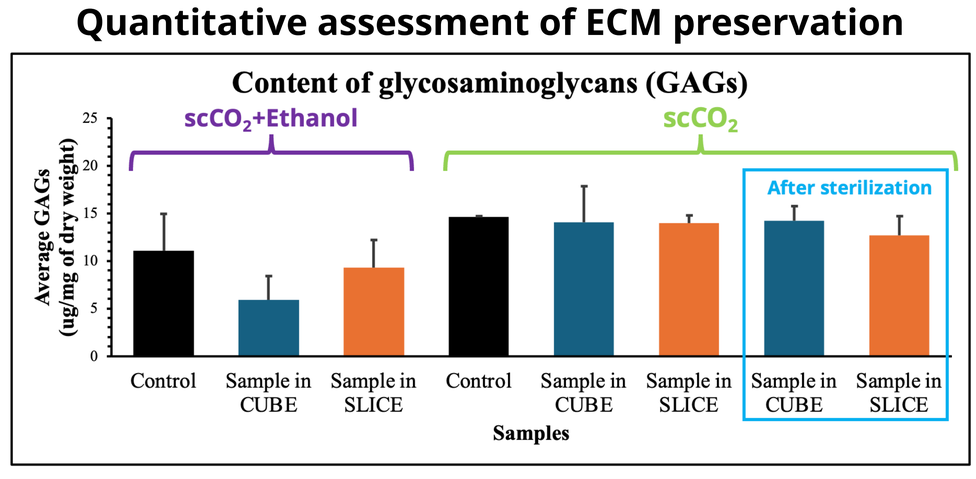
DNA reduction rate	Sample in ***CUBE***	Sample in ***SLICE***
$scCO_2$	**71.83%**	**66.41%**
$scCO_2$+Ethanol	**66.36%**	**69.29%**

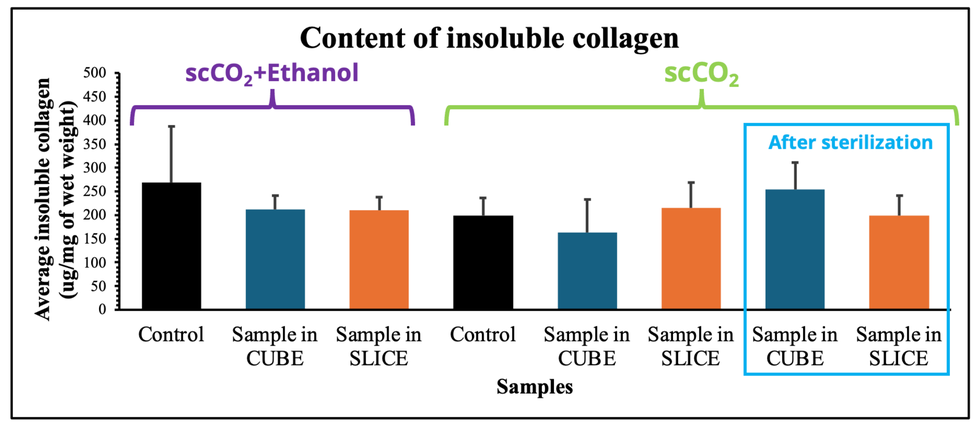
Fourier-transform infrared spectroscopy (FTIR)



FTIR analysis indicated **ECM preservation in both protocols**, although ***minor conformational changes*** may have occurred. Notably, in **protocol with $scCO_2$+Ethanol**, spectral features observed between **1040--1160 cm^{-1}** suggest the presence of **residual ethanol** in meniscal tissue, corresponding to **C–O bond vibrations**.

Quantitative assessment of ECM preservation





Conclusions

The protocol involving **9 cycles of pure $scCO_2$**, followed by freeze-thaw and washing steps, demonstrated **enhanced efficacy for decellularization** of porcine meniscal tissue. **Sample thickness did not significantly affect DNA removal efficiency** in either protocol. Furthermore, the use of **ethanol as a co-solvent** introduced **a risk of residual ethanol retention** in the tissue. **Enhanced preservation of ECM components** was also observed with the **pure $scCO_2$ approach**. Additionally, the **sterilization process** did not impact the preservation of ECM components.

References

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Acknowledgements

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