Decellularised Pleural Membranes in Pulmonary Regenerative Medicine

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Research aim

Develop tissue-engineered porcine pleural membrane grafts for the clinical management of prolonged alveolar air leaks

Clinical focus

Prolonged alveolar air leaks (PAL)

- Sustained air leaks > 5 days
- 8% - 26% post-surgical incidence
- Secondary pulmonary and cardiovascular complications
- Revision surgeries
- Extended length of hospital stays (LOS)

Current management

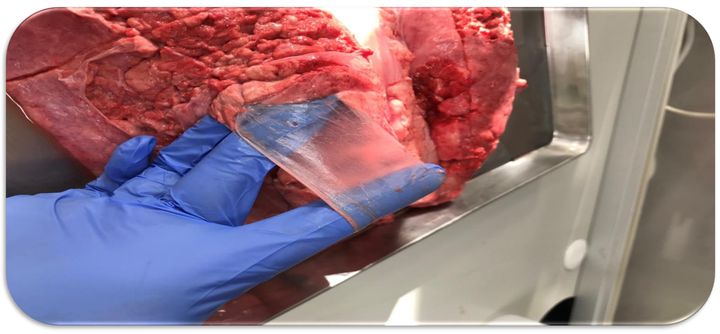
- Extended chest tube duration
- Intra-operative surgical measures
- Application of biomechanical sealants
- Chemical pleurodesis

Clinical need

- Reduced PAL incidence
- Reduced risk of comorbidities and LOS
- Induce spontaneous lung tissue regeneration
- Expedite post-surgical recovery

Methodology

Pleural membrane (PM) excision



PM Decellularisation

Freeze-thaw cycles at (-80°C / room temperature)

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Treatment with decellularisation buffer (0.5% sodium deoxycholate + 1% v/v Triton X-100 in 10mM tris-buffer, pH 7.6 + 30µg/ml DNase I) for 48hours

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Periodic washes with phosphate buffered saline (every 12 hours for 48 hours) followed with distilled water (every 8 hours for 48 hours)

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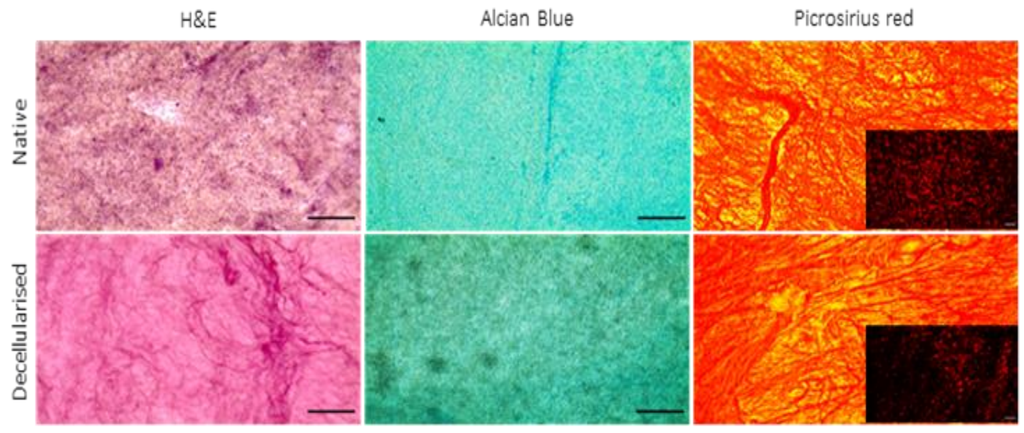
Decellularised PM stored at 4°C for 2 weeks

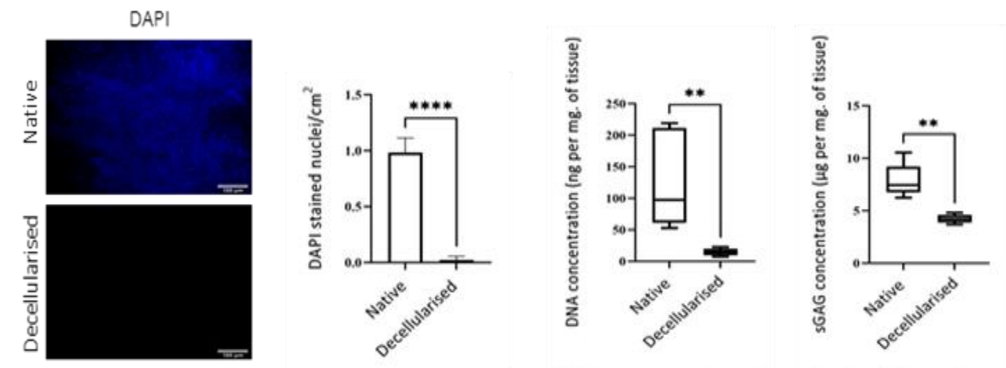
Decellularised PM characterisation

- Histology and DAPI staining
- Quantitative bioassays
- Biomechanical testing
- Cytotoxicity and recellularisation assays

Results



Histological features observed in native vs decellularised PM with H&E staining exhibiting visible reduction in nuclei in the decellularised PM. Alcian blue and Picrosirius red staining for sulphated glycosaminoglycans (sGAG) and collagen respectively, showed minimal disruption to the specific core extracellular matrix components following PM decellularisation. (Scale bar = 100 µm)

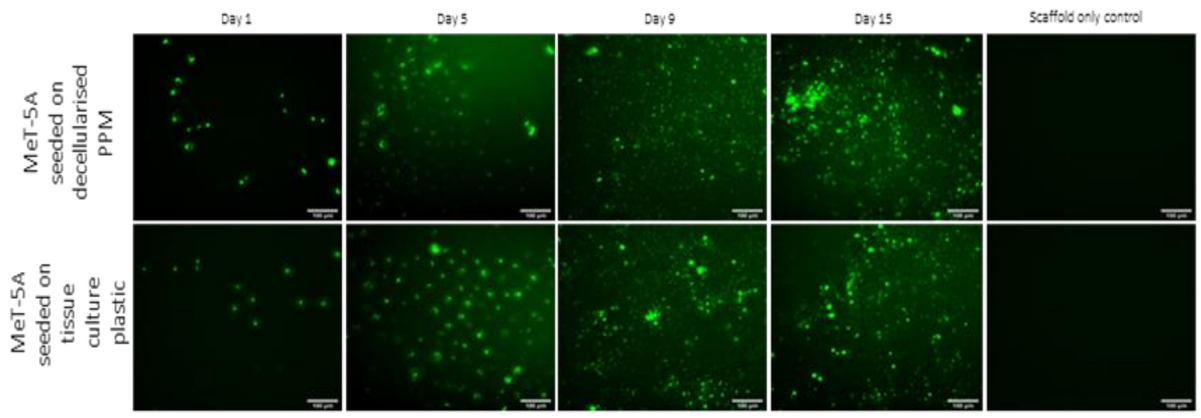


Nuclear staining with DAPI exhibiting a statistically significant reduction (****$p < 0.0001$) in nuclei in the decellularised PM. Residual DNA content determined using PicoGreen assay was less than 50 ng per mg. dry weight of decellularised PM, conforming to the established criteria for efficient decellularisation (**$p < 0.005$). sGAG content determined using the Dimethyl methylene blue (DMMB) assay showed a significant reduction in the decellularised PM vs native tissue (**$p < 0.05$)

Mechanical properties			
Sample	Mean membrane thickness (µm)	Mean Youngs modulus (kPa)	Mean Ultimate tensile strength (kPa)
Native PPM	147 ± 27	9259.5 ± 2079	33122 ± 1837
Decellularised PPM	195 ± 11*	12782.7 ± 3874	24551.6 ± 2983*

Data represented as Mean ± SD, n = 3. *$p < 0.05$ vs native PPM control

Biomechanical characterisation of decellularised PM versus native pleural membranes as control



Attachment, viability and proliferation of labelled MeT-5A cells (human mesothelial cell line) seeded on decellularised PM assessed using fluorescence imaging at defined time points. Scale bar = 100 µm.

Summary

- Optimal pleural membrane decellularisation with the adopted protocol.
- Significant reduction in cellular and nuclear material in decellularised PM
- Minimal disruption of gross extracellular matrix morphology and core structural composition.
- Significant increase in decellularised PM thickness with a reduction in ultimate tensile strength.
- Native PM viscoelasticity, however, conserved in the decellularised PM.
- Scaffold biocompatibility and minimal cytotoxicity exhibited by the decellularised PM, promoting attachment and proliferation of seeded MeT-5A cells at progressive time points in the study.

Conclusion

Our pilot study represents a step forward in deriving bioactive pleural grafts in the form of decellularised PM. Studying the recellularisation dynamics of the cell-seeded scaffolds using primary mesothelial cultures, will underpin our future research towards developing proof of concept for application of the relatively unexplored decellularised pleural membranes in pulmonary regenerative medicine.