Decellularised pleural patches for management of prolonged alveolar air leaks

Trisha Vikranth[1], Dr Tina Dale[1], Prof Nicholas Forsyth[1]

[1]School of Pharmacy and Bioengineering, Keele University, United Kingdom

Research aim

Optimise a decellularisation and characterisation protocol for porcine sourced pleural membranes for its application as a tissue engineered therapeutic alternative in the clinical management of PAL

Clinical Focus

Prolonged alveolar air leaks (PAL)

- Alveolar air leaks > 5 days
- 8% - 26% post-surgical incidence
- Secondary pulmonary and pleural infections
- Extended length of hospital stays (LOS)

Risk factors

- Lung resections and volume reduction surgeries
- Chronic lung pathology
- Physical trauma
- Chemo/radiotherapy

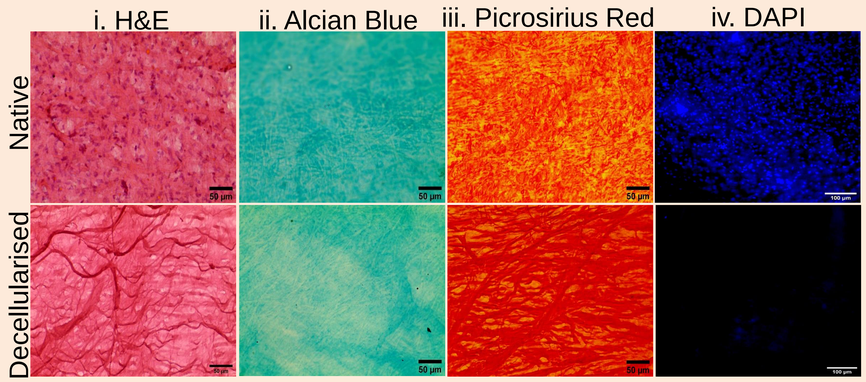
Current management

- Conservative (chest tube insertion)
- Intra-operative (staples and sutures)
- Sealants (biological/synthetic)

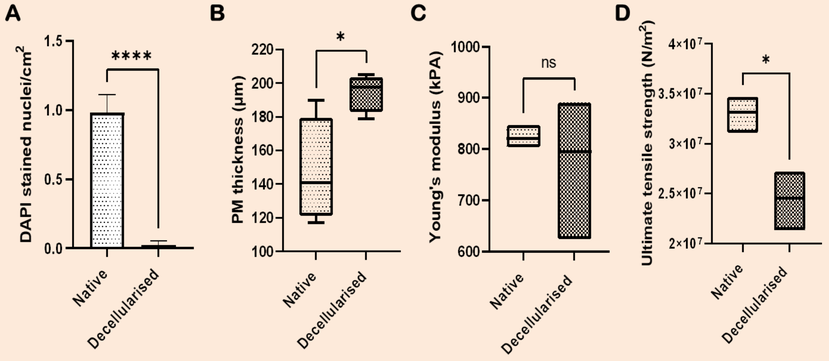
Clinical need

- Reduced PAL incidence
- Reduced risk of co-morbidities and LOS
- Spontaneous pleural membrane regeneration

Main Findings



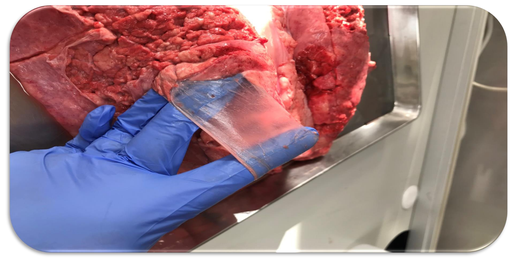
Histological analysis of Decellularised vs Native PM. (i) H&E staining showed visible reduction in purple stained nuclei in the decellularised PM. (ii) Alcian blue for GAGs and (iii) Picrosirius red for collagen exhibited comparable staining in the decellularised PM, indicative of minimal alteration to the composition and organisation of the native pleural ECM. (iv) Fluorescence imaging of DAPI stained nuclei showed visible reduction in number in the decellularised PM.



Quantitative assessments of the effect of decellularisation on (A) Nuclear membrane integrity. Significant reduction in DAPI stained nuclei in the decellularised PM (****p< 0.001). (B) Membrane thickness. Significant increase in membrane thickness post-decellularisation (*p < 0.05). (C) Mechanical integrity. Estimated Young's modulus comparable in decellularised PM to its native counterpart (ns = non-significant, p > 0.05). (D) Significant decrease in ultimate tensile strength in the decellularised PM (*p < 0.05).

Methodology

PM excision



Decellularisation

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

↓

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

↓

Periodic washes with phosphate buffered saline (48 hours) followed with distilled water (48 hours)

↓

Decellularised PM stored at -4˚C for 2 weeks

Characterisation

- Histology studies (H&E, collagen, glycosaminoglycans GAGs)
- Nuclear membrane integrity study (DAPI staining)
- Membrane thickness estimation
- Uni-axial tensile testing (mechanical)

Conclusion

Our pilot study represents a step forward in deriving clinically relevant bioactive ECM scaffolds in the form of decellularised pleural membranes. Future work involves biomolecular assays, ultrastructural studies and assessing the recellularisation potential of the derived scaffolds.

t.vikranth@keele.ac.uk