PP-152 Tissue engineered lungs in rats and nonhuman primates

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Objective

Decellularization with *en bloc* recellularization is a promising strategy for the creation of tissue engineered lungs. Our study focused on the *ex vivo* recellularization of rat lungs and histological evaluation in hopes of transferring the protocol to a nonhuman primate (NHP) model.

Material and methods

Eighteen adult male Lewis rats, weighting 180±16 g, were used to decellularize and recellularize rat lungs. All animal procedures conformed to ethical requirements. Native rat lungs were mounted in bioreactors and decellularized according to our modified protocol: air tracheal ventilation and antegrade perfusion *via* the pulmonary artery for 23 hours with decreased concentration and timing of ionic detergent (1.5 hour of 1% sodium deoxycholate) and non-ionic detergent (1 hour of Triton X -100). *En bloc* lung recellularization was carried out by oxygenated medium with bone marrow - derived mesenchymal stem cell perfusion in a customized bioreactor for 7 days. Native (n=6), decellularized (n=6) and recellularized (n=6) rat lungs were evaluated by histological staining and immunohistochemical characterization for remaining nuclei, extracellular matrix proteins (ECM) and glycosaminoglycans (GAG) after processing with specialized kits. DNA quantification was performed using a spectrophotometer BioDrop (Biochrom Ltd, Cambridge, UK). Cell proliferation assays and live/dead cell staining were used to measure cell viability and metabolic activity on the reseeded lung scaffold. For the NHP model, three intact heart and bilateral lung blocs were isolated from male rhesus macaques (Macaca mulatta). These lungs were decellularized using the same detergent-based perfusion protocol; however, the time was increased to 48 hours. Histological, immunohistochemistry methods and DNA quantification proved the efficiency of decellularization.

Results

After decellularization, the rat lungs showed no remaining nuclei and well preserved matrix architecture after histological evaluation. Immunohistochemical staining proved ECM protein (positive staining for collagen I, collagen IV, laminin, elastin, fibronectin) and GAG preservation. Total DNA decreased from 581.03±18.42 ng/µg in native lung tissue to 24.73± 0.82 ng/µg in the decellularized group ($p=0.00467$). Cell proliferation assay and live/dead cell staining after recellularization indicated the scaffold's ability to support cell adhesion and proliferation ($p=0.0024$). Haematoxylin and eosin staining demonstrated the presence of reseeded cells on *en bloc* recellularized lungs. After recellularization, the rat lung cytoskeleton stained positive for actin and VEGF; cell proliferation was indicated by positive nuclei staining by Ki-67. Using a similar protocol for the decellularization of NHP lungs, anatomical properties of the native tissue were maintained, ECM proteins and GAG were preserved and no evidence of intact nuclei or cell components was detected. 89% of cellular DNA was removed after decellularization ($p=0.0001$).

Conclusion

A detergent-based perfusion decellularization protocol can be used to obtain scaffolds for rat and NHP lung recellularization.