

Landmark papers in respiratory medicine

Bioartificial lungs based on de- and recellularisation approaches: a historical perspective

For patients with end-stage respiratory diseases, such as COPD, interstitial lung diseases and cystic fibrosis, lung transplantation remains the only treatable option. However, due to increasing demand and limited availability of donor lungs, risk of complications such as acute and chronic rejection, and adverse effects of immunosuppressive treatments, this is not an alternative for the majority of this patient group [1, 2]. To meet the rising clinical demand new strategies to increase the number of available lungs for transplantation are needed [2]. One such strategy involves creating a functional lung *ex vivo* using different de- and recellularisation approaches. In this article, we will provide an overview of three landmark studies on bioartificial lungs published during 2010 that set the base for the direction of this relatively young field.

Decellularised murine lung matrix bioreactor system

In this pioneering study, PRICE *et al.* [3] utilised a previously described detergent-based tissue decellularisation protocol and scaled it up for whole mouse lungs. Perfusion of detergents (Triton-X, Na deoxycholate) and other reagents through both the trachea and vasculature resulted in decellularised lungs that maintained their overall shape and both macroscopic and microscopic structure. Furthermore, the decellularised lungs were able to be ventilated in a bioreactor system for up to

14 days. Histological evaluation demonstrated that the decellularised mouse lungs had no remaining nuclei and that the extracellular matrix (ECM) was mostly visually intact. However, quantification of laminin and elastin in homogenates of the decellularised lungs demonstrated a significantly lower level compared to native lungs. Collagen levels were similar to those found in native lungs, and no matrix metalloprotease (MMP) activity was detected (MMP-2 and MMP-9). Levels of sulfated glycosaminoglycans (GAGs) were also significantly decreased in decellularised lungs compared to levels in native lungs [3].

To evaluate if lung cells could repopulate the decellularised lung, 3 million fetal mouse lung cells were infused. Cryosections were taken after 7 days of ventilation, and CK18 (epithelial cells), pro-SP-C (ATII cells) and DAPI (nuclei) positive cells were detected in alveolar areas. No cells expressing aquaporin-5 (ATI cells), CD11b (macrophages), CD31 (endothelial cells), CCSP (Clara cells) or vimentin (fibroblasts) were found in the repopulated lung [3]. Assessment of mechanical properties of the decellularised lungs was performed using pressure-volume measurement tests obtained with a FlexiVent device. Compared to the native control lung, decellularised lungs had decreased compliance and increased resistance and elastance. No improvements were obtained after 7 days of ventilation or after repopulating the lung with 1 million fetal lung cells. Taken together, in this pioneering study PRICE *et al.* [3] decellularised whole mouse lungs that were kept in a bioreactor system

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A discussion of three landmark studies on bioartificial lungs published during 2010 that were instrumental in invigorating the lung regenerative medicine field <https://bit.ly/31qQAEa>



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in which they successfully repopulated the murine matrix with fetal alveolar type II cells.

Tissue-engineered rat lungs for *in vivo* implantation

In this study, PETERSEN *et al.* [4] used a different detergent (CHAPS)-based perfusion protocol to decellularise whole rat lungs. Similar to PRICE *et al.* [3] the lung was cannulated and the decellularisation solution was administered through both the trachea and the vasculature. Using this method, the alveolar micro-architecture, barrier function and tissue mechanics were found to be preserved. Although the overall macro- and microscopic lung architecture was intact, histological evaluations of the decellularised tissue showed that while collagen was preserved, elastin was partly depleted (60% removed), and more than 90% of the sulfated GAGs were removed by the decellularisation process [4].

To test the functional properties of the decellularised whole rat lung, the lung was mounted in a bioreactor designed to mimic the fetal lung environment. Culture media was perfused into the pulmonary artery and a negative pressure inflation was obtained by inhalation of media *via* a breathing loop. Next, neonatal lung epithelial cells were administered *via* the airway compartments and microvascular lung endothelial cells *via* the pulmonary artery to assess repopulation. The epithelial cells were observed to replicate with minimal apoptosis observed markers. Moreover, the negative pressure ventilation in the bioreactor was demonstrated to have beneficial effects on the repopulated epithelial cells, including enhanced survival in distal alveoli and in clearance of secretions. Ventilation with air increased the number of type I alveolar epithelial cells and ciliated columnar epithelial cells compared to ventilation with media. Compliance testing demonstrated that both acellular lungs and repopulated lungs had lower compliance compared to native lungs. However, the ultimate tensile stresses and the overall stress-strain relationships were similar between the three groups. To test if the de- and recellularisation protocols reported in this study worked on human lung tissue, human lung segments were decellularised for up to 6 h. Histological evaluation of the sections demonstrated complete cellular removal and an intact alveolar architecture. Next, segments were repopulated with human cord-blood endothelial progenitor cells and human lung epithelial carcinoma cells. Endothelial cells adhered to the vasculature and the epithelial cells to the alveolar surfaces and, therefore, the authors concluded that these protocols were applicable to human lung tissue [4].

Finally, the authors wanted to test if the engineered rat lungs were functional for short time periods (45–120 min) by implanting the lungs into a syngeneic rat model (orthotopic left lung).

The repopulated lungs were found to be easily suturable to the recipient, no air leak was observed, and all lungs became perfused. The repopulated lungs were inflated with air, but the levels were less compared to the native lung. Histological evaluation demonstrated modest bleeding into airways. The partial pressure of oxygen increased in both the pulmonary artery and the left pulmonary vein; however it did not reach the values obtained in native lung. Although, the partial pressure in the bioengineered lung did not reach the values obtained in the native lung, the haemoglobin saturation was 100% for both the repopulated lung and the native lung. Taken together, in this study PETERSEN *et al.* [4] repopulated decellularised rat lung with neonatal lung cells and showed that the repopulated lung participated in gas exchange for a short period of time.

Orthotopic transplantation of a bioartificial rat lung

Using another different detergent perfusion protocol (0.1% of sodium dodecyl sulfate), OTT *et al.* [5] successfully decellularised whole rat lungs. No evidence of remaining nuclei (except for scattered nuclear remnants in cartilaginous rings) or intracellular elements were found. Importantly, ECM proteins, as well as the matrix architecture of airways and vasculature, were found to be preserved. Using a similar approach, OTT *et al.* [5] evaluated the applicability of this protocol to human-sized organs including sheep, porcine and baboon lungs successfully.

To test if the decellularised whole lung could be repopulated and maintained *ex vivo*, a decellularised rat lung was repopulated with human umbilical cord endothelial cells (through the vasculature) and kept in a bioreactor system designed to simulate the fetal lung environment just prior to the transition to air breathing. After 5 days in culture, endothelial cells were demonstrated to be spread and engraft throughout the scaffold. Next, OTT *et al.* [5] combined the repopulation of endothelial cells (through the vasculature) with carcinomatous human alveolar basal epithelial cells (through the trachea). The repopulated scaffolds were maintained for 9 days, after 5 days of culture epithelial cells formed a monolayer, but after 6 days of culture cells started to form multilayer and obliteration of airways and loss of surface area was observed [5].

Decellularised cadaveric lungs repopulated with rat fetal lung cells and human umbilical cord endothelial cells was cultured under physiological conditions for up to 9 days. The architecture of large and small conducting airways, alveoli and vasculature was preserved, and the repopulation was reported to be successful. To test functional properties of the repopulated lungs the fluid ventilation was substituted with dry ventilation after 5 days in culture. The gas exchange of the fetal

lung cells and human umbilical cord endothelial cells repopulated lungs matched the levels of native lungs regarding vital capacity and compliance. Finally, the bioengineered lungs were evaluated *in vivo* after orthotopic transplantation into normal rats. The transplantations were successful, and rats were extubated and maintained without ventilation support for 6 h. However, over the following several hours the rats developed pulmonary secretions without blood. Graft analysis demonstrated vascular and bronchial anastomoses and appeared oedematous, moreover high inspiratory pressure were needed for complete expansion. Taken together, in this proof of concept study OTT *et al.* [5] repopulated a cadaveric lung with endothelial and epithelial cells with a functional short-term gas exchange both *in vitro* and *in vivo*.

Lessons learned from the landmark studies

Although decellularisation of whole lung was first described in 1986 [6], these three landmark papers published in 2010 have been essential for reinventing the regenerative medicine field and have stimulated intensive investigation [3–5]. Common in these studies was that they all administered the decellularisation solutions through both the airway and the vascular system. Retention of both macro- and micro architecture was reported. However, degradation of laminin, elastin, and sulfated GAGs were found after the decellularisation process, although the degree of degradation was different between the different studies. Most importantly, these proof-of-concept studies demonstrated short-term gas exchange following implantation into rodents [5]. Although haemorrhage and thrombus formation were observed in the study by PETERSEN *et al.* [4] and substantial oedema was observed by OTT *et al.* [5], these ground breaking studies set the stage for the direction of the field.

Challenges and opportunities in the field: where do we stand?

Studies on creating a bioartificial lung based on de- and recellularisation approaches demonstrated promising results in the beginning of 21st century. However, the lack of satisfactory repopulation of functional cells and proper long-term gas exchange has hampered the progression of the field. The lung is a very complex mechanical organ composed of various cell types and ECM components [7]. The degradation of proteoglycans and their GAGs after the decellularisation process is an important observation that might be one of the explanations behind the lack of proper cell repopulation and tissue functionality [4, 6, 8]. GAGs are known to be involved in ECM organisation and assembly, as

well as in remodelling processes during normal homeostasis [9]. Moreover, GAGs are important in the cell-ECM interactions and play an important role in directing cell behaviour [9, 10].

Moreover, a functional vasculature in bioengineered lungs is critical for proper graft function. Given the essential need for a functional and intact vasculature, DORRELLO *et al.* [11] developed an airway-specific rat model in which they aimed to preserve the pulmonary vasculature and the ECM components. By histological staining the authors demonstrated removal of airway epithelium, while preserving a portion of parenchymal lung cells including endothelial cells (43%) and interstitial cells (32%) compared to the control. Importantly, the maintained blood vessels responded to both vasoconstrictors and vasodilators after the decellularisation process. Except for loss of epithelial cell-associated GAGs and decreased compliance, the lung architecture and matrix proteins were demonstrated to be fairly preserved [11]. In a recent study by NICHOLS *et al.* [12], the authors explored *in vivo* implantation of decellularised pig lungs seeded *ex vivo* with a variety of cells but without anastomosing the implant to the host vasculature. These studies are emblematic of both the advances and the challenges involved in recreating or preserving a functional pulmonary vasculature.

Another challenge is that the lung is a very complex organ consisting of numerous cell types. It is critical to understand which cells are most necessary for repopulating the decellularised lung. Repopulation could potentially be done with differentiated primary human cells, however this will require usage of multiple cell types either at the same time or sequentially. An alternative would be to repopulate the lung with progenitor cells or induced pluripotent stem cells and stimulate differentiation. The next important step will then be to guide the cells to their correct location within the scaffold. 3-dimensional printing and bioprinting are techniques that would be ideal to address these concerns, but due to technical limitations it has been a slow progression [13]. Further investigations are needed to develop bioreactor technologies designed to provide the lung with oxygen, nutrition and mechanical ventilation, as well as real-time lung function measurements [14]. In addition, some practical issues for translation into clinical settings involving potential immunogenicity, sterilisation protocols, evaluation methods, strategies to forecast outcomes, and the overall practicality of this approach, needs to be solved.

Summary

In the light of these studies, the enthusiasm for bioengineering lung using de- and recellularisation approaches was raised and the number of studies significantly increased in this fascinating field. Since

2010, several combinations of cells have been tested, de- and recellularisation protocols have been refined, and more advanced bioreactors have been developed [13–17]. However, the field is still struggling with the lack of long-term gas exchange and a successful recellularisation of the lung tissue with functional cells. Despite these limitations, there has been significant progression in this relatively young field with improved techniques and

bioreactor devices, and hopefully ongoing studies will bring improved transplantable bioartificial lungs and hope for the future. Another possibility for the future of the artificial lung could potentially be to combine a gas exchanger with a pump creating a small device. This artificial lung could then potentially put human lung transplantations aside, avoiding many of the complications related to organ transplantation.

Affiliations

Sara Rolandsson Enes¹, Daniel J. Weiss²

¹Dept of Experimental Medical Science, Faculty of Medicine, Lund University, Lund, Sweden. ²Dept of Medicine, 226 Health Science Research Facility, Larner College of Medicine, University of Vermont, Burlington, VT, USA.

Conflict of interest

S. Rolandsson Enes has nothing to disclose. D.J. Weiss has nothing to disclose.

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