Acellular human lung scaffolds to model lung disease and tissue regeneration

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ABSTRACT Recent advances in whole lung bioengineering have opened new doors for studying lung repair and regeneration *ex vivo* using acellular human derived lung tissue scaffolds. Methods to decellularise whole human lungs, lobes or resected segments from normal and diseased human lungs have been developed using both perfusion and immersion based techniques. Immersion based techniques allow laboratories without access to intact lobes the ability to generate acellular human lung scaffolds. Acellular human lung scaffolds can be further processed into small segments, thin slices or extracellular matrix extracts, to study cell behaviour such as viability, proliferation, migration and differentiation. Recent studies have offered important proof of concept of generating sufficient primary endothelial and lung epithelial cells to recellularise whole lobes that can be maintained for several days *ex vivo* in a bioreactor to study regeneration. In parallel, acellular human lung scaffolds have been increasingly used for studying cell–extracellular environment interactions. These studies have helped provide new insights into the role of the matrix and the extracellular environment in chronic human lung diseases such as chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. Acellular human lung scaffolds are a versatile new tool for studying human lung repair and regeneration *ex vivo*.

Introduction

Chronic lung diseases such as chronic obstructive pulmonary disease are currently the third leading cause of death worldwide. Other chronic respiratory diseases, such as interstitial lung disease (ILD) or pulmonary arterial hypertension, and genetic conditions, such as α_1-antitrypsin disorder and cystic fibrosis, are devastating lung diseases with no cure. At end-stage disease, transplantation is the only option. In addition to a shortage of suitable donor lungs, lung transplantation is further complicated by high rates of both acute and chronic rejection. As a result of these challenges, the current 5-year survival rate is 50% [1]. Despite significant efforts to improve pre- and post-operative care, the average survival rates have remained relatively unchanged over the past two decades [1]. New approaches are urgently needed for these patients.

Ex vivo lung tissue engineering has recently emerged as a potential option for increasing the amount of tissue available for transplantation [2]. Lung tissue engineering follows successful clinical approaches using

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other acellular tissues such as skin [3, 4]. In an ideal scenario, cells would be harvested from the intended transplant recipient, seeded onto a scaffold *ex vivo*, grown in a bioreactor until the tissue reaches maturity, and finally transplanted (figure 1) [2]. One approach being explored in preclinical models uses a biological scaffold (also called an acellular scaffold) that is derived by decellularising native lung tissue. The goal of any decellularisation protocol is to remove the native cells while preserving both the micro- and macroarchitecture of the organ, as well as the extracellular matrix (ECM) composition and tissue structure [3]. Numerous studies have shown that acellular scaffolds can be derived from a variety of species, including rodents, non-human primates, pigs and humans [5–14].

In parallel, techniques have been developed to generate acellular lung scaffolds originating from animals and also from patients with existing lung diseases [6, 15–21]. These acellular scaffolds retain structural and compositional characteristics of the diseased organs from which they were derived. When cells are reseeded onto these scaffolds, they adopt phenotypes similar to those observed *in vivo* and thus have emerged as new three-dimensional model systems for studying human disease *ex vivo* and in particular, cell–matrix interactions.

In this mini-review, we focus on the use of acellular human lung scaffolds as models to study lung repair and regeneration and recent advances in *ex vivo* lung regeneration for eventual transplantation using acellular human lung scaffolds.

Methods of de- and recellularisation

The first report of generating and using acellular human lung tissue to study cellular behaviour was reported in 1986 by Lwebuga-Mukasa *et al.* [22]. They decellularised resected segments of human lung tissue from pneumonectomy using a detergent based protocol in order to study the effect of the basement membrane on the morphology and function of type II cells. Following decellularisation, they recellularised the scaffold with freshly isolated rat alveolar type II cells and observed that these cells took on a flattened morphology and lost their lamellar bodies and surface microvilli. They noted that these cells survived up to 8 days *ex vivo* and underwent morphological flattening and loss of lamellar bodies in this *ex vivo* system in a similar manner to *in vivo* alveolar type II to type I differentiation. Interestingly, these morphological changes did not happen as rapidly when cells were cultured on acellular human amniotic basement

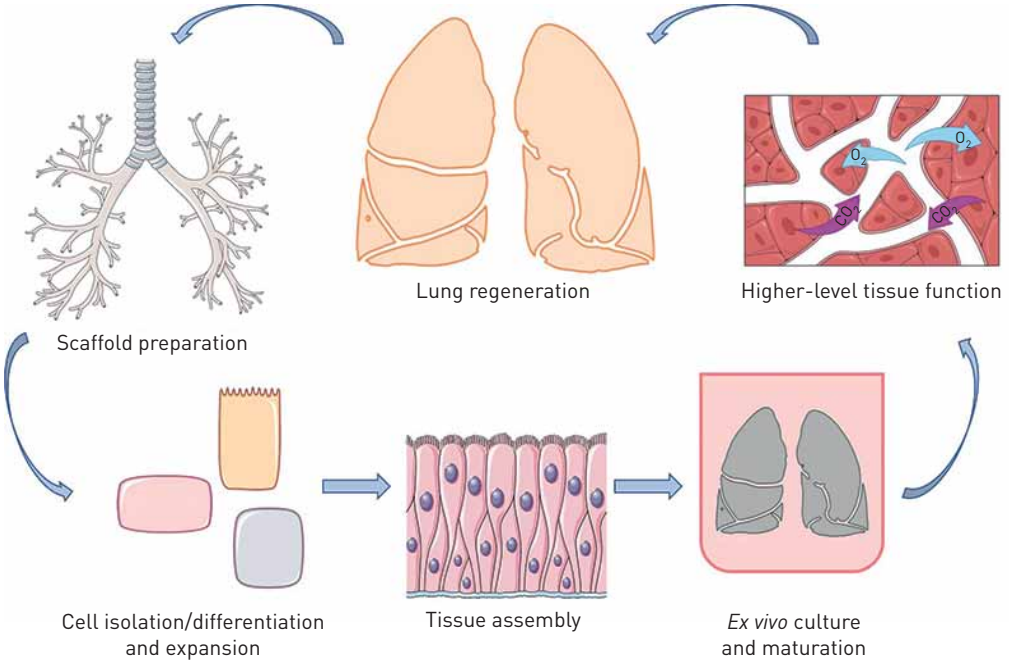

FIGURE 1 Schematic outline of the steps in whole lung regeneration. Acellular lung scaffolds are first prepared from donated organs otherwise unsuitable for transplantation. Necessary cell populations must be isolated and/or differentiated and expanded to adequate numbers for scaffold recellularisation. Following delivery of the required cell populations to the correct anatomical positions within the scaffold, the cells must be matured to form organised tissues. This process includes *ex vivo* culture of the regenerating lung, within specialised bioreactors, which can recapitulate native organ mechanics. Finally, higher-level tissue function, including fluid balance and gas exchange, can be tested both *ex vivo* and following *in vivo* implantation in large animal transplantation models. Images reproduced and modified from Servier Medical Art with permission.

membranes decellularised in a similar manner [23]. Thus, the residual extracellular environment in which these rat alveolar type II cells were cultured had a significant influence on their *ex vivo* behaviour. It took nearly 25 years before acellular lung scaffolds from human lungs were used as *ex vivo* models of lung repair and regeneration again.

The techniques used for generating acellular lung scaffolds differ by species and disease status. The majority of protocols rely on a series of detergents used to lyse and remove cells and cell debris, while retaining the ECM composition and structure. There are two main approaches that have been used for decellularisation of lung tissue: 1) perfusion based decellularisation, where decellularisation solutions are perfused through the vasculature and/or airways of the lungs; or 2) immersion based decellularisation, where segments of human lungs are submerged in decellularisation solutions with or without agitation (figure 2). While up-scaling the processes developed in mice to large animal and human lungs has been challenging, several successful protocols now exist for obtaining whole acellular human lung scaffolds [5–11, 24]. Whole acellular lung scaffolds are not needed for all *ex vivo* models. In fact, for some studies, only certain regions of the lung are of interest. Therefore, techniques have also been developed for decellularisation using immersion based techniques in resected segments of parenchymal lung and bronchi (figure 2) [15, 25, 26].

There are currently no uniform criteria for what constitutes a decellularised lung. The majority of groups utilise the minimal criteria put forth by Crapo *et al.* [3] to define a decellularised tissue: 1) lack of visible nuclei by histological and immunofluorescence staining, 2) <50 ng of double-stranded DNA per mg tissue dry weight, and 3) DNA fragments <200 bp. However, these criteria do not take into account the mechanical properties of the tissue, sterility of the tissue, the ECM composition or the cytocompatibility of the resulting scaffold. All of these criteria will have an impact on the behaviour of cells re-introduced to the scaffold.

Cytocompatibility is a necessary criterion for any recellularisation study. Owing to difficulties in removing detergents from lungs of larger species, including human lungs, using perfusion based decellularisation approaches, several groups have also evaluated detergent levels during and following decellularisation [5, 24, 27]. This is particularly important to monitor as different cell types have been found to have different thresholds for detergent-induced toxicity [27]. Therefore, differences in residual detergents, which may arise from technical differences (*e.g.* size of the lung) or differential retention of detergents due to disease state or species, may affect initial cell binding, cell survival and subsequent proliferation. Techniques that do not use detergents may also have residual components that may have an impact on cell survival; thus, each new protocol needs to be thoroughly investigated with regards to cytocompatibility. Achieving both short- and long-term sterility is critical for any tissue engineered scaffold, including the lung. Several studies have evaluated the impact of sterilisation procedures on lung architecture and its impact on recellularisation in animal models and human scaffolds [5, 9, 28, 29]. Thus, additional criteria for sterility and cytocompatibility of acellular scaffolds could be as follows: 1) a sterility assurance level of $<10^{-6}$ in the acellular scaffold following any sterilisation procedure [29], and 2) $\geqslant$70% of cells should survive effluent testing from whole organs or on slices [27]. Fulfilment of both of these criteria is necessary for successful recellularisation.

ECM composition is also an important end-point following decellularisation. Mass spectrometry proteomics has been used to examine differences in the protein composition of acellular human scaffolds using different decellularisation parameters such as choice of detergent or perfusion flow rate [5, 9, 25, 30, 31]. Interestingly, acellular scaffolds derived from normal human lungs retain unique protein compositions [9], independent of the flow rate used for decellularisation. Thus, if a standard decellularisation method is used across a patient cohort, acellular human lung scaffolds can be used to study the heterogeneity that exists between normal patients.

To date, several different methods have been developed for reintroducing cells to acellular human lung scaffolds (termed recellularisation) (figure 2). Following the successful development of techniques to decellularise whole human lungs using perfusion based techniques or segments of human lungs using immersion based techniques, initial studies focused on testing the cytocompatibility of the resulting scaffold. In these studies, cells were either seeded onto thin slices of acellular lung scaffolds or seeded into small segments of acellular lung scaffolds, and initial cell attachment, survival and proliferation were examined over time [5–7, 11, 25, 32]. Following the success of these techniques, methods to selectively recellularise through the vasculature or airways of resected segments were then developed [9, 33]. This technique allows the study of more specific cell–ECM interactions (*e.g.* endothelial basement membrane *versus* epithelial basement membrane), rather than relying on stochastic cell adhesion when cells are exposed to the whole scaffold. Regardless of the technique used for recellularisation, these early studies importantly confirmed that the techniques used for decellularisation yielded cytocompatible scaffolds. Acellular human lung parenchyma or bronchial scaffolds [26] have been shown to provide support for cell

survival and proliferation of several different types of primary and immortalised cell lines for up to 35 days *ex vivo* (table 1), making them suitable models for studying both short- and longer-term processes.

In addition to recellularisation into segments or onto thin slices, techniques to homogenise and solubilise the ECM derived following decellularisation have been developed and allow the study of cell–ECM

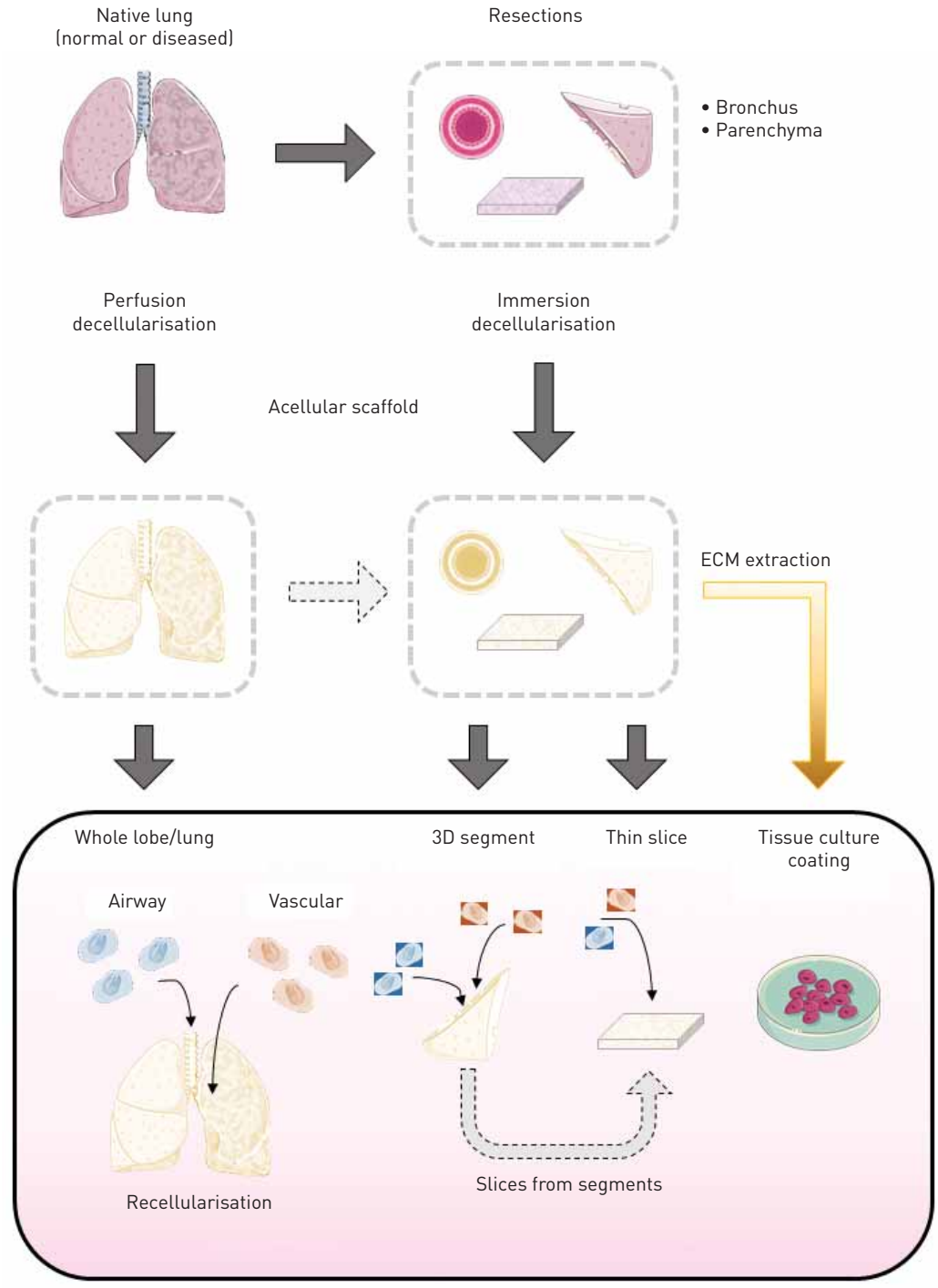

FIGURE 2 Overview of techniques to generate acellular human lung scaffolds for modelling lung disease, repair and regeneration. Acellular scaffolds may be obtained from normal or diseased human lungs using either perfusion or immersion based decellularisation. Following successful decellularisation, cells may be reseeded into whole lungs or lobes, seeded into small three-dimensional (3D) segments, or seeded onto thin slices. Recellularised small 3D segments may be left intact or further processed into thin slices. Alternatively, extracellular matrix (ECM) may be extracted from acellular human lung tissue and used to coat cell culture dishes. Images reproduced and modified from Servier Medical Art with permission.

TABLE 1 Comprehensive list of studies using acellular normal or diseased human lung tissue

Year	Human lung status	Recellularisation technique	Cell type(s)	Significant finding	[Ref.]
1986	Normal	Thin slices	rATII	Acellular scaffolds can be produced from human lungs using detergent based cell removal; rATII cells adhere to human lung scaffolds	[22]
2010	Normal	Dissected segment incubated with cells	A549, endothelial progenitor cells	Acellular lungs support recellularisation	[7]
2012	Normal/IPF	Thin slices	HLFs	IPF scaffolds induce α-SMA expression in HLFs	[6]
2013	Normal	Thin slices	iPSC-ATII, ATII	iPSC-ATII cells adhered to acellular lung scaffolds; subpopulation differentiated to ATI phenotype	[34]
2013	Normal	Thin slices	MRC-5, SAECs	Acellular lung scaffolds decellularised using immersion techniques supported cell viability	[25]
2013	Normal	Dissected segment injected with cells	mESCs, HFLCs, pBM-MSCs, AECs	Acellular lung scaffolds support cellular attachment; 1% SDS minimises T-cell activation	[11]
2014	Normal	Thin slices; single lobe recellularisation	HUVECs, SAECs, PAECs	Cell survival for 3 days in an acellular perfused upper right lung lobe recellularised with PAECs	[5]
2014	Normal	Thin slices	iPSC-ATII	iPSC-derived ATII cells can repopulate acellular lung scaffolds and recycle surfactant	[32]
2014	Normal	Thin slices	iPSC-derived ventralised endoderm	Enhanced NKX2-1 specification on ECM	[35]
2014	Normal	Thin slices and rotating bioreactor	iPSC-ATII, ATII	Rotating bioreactor culture at air–liquid interface enhanced ATII to ATI differentiation	[36]
2014	Normal	Thin slices	BM-MSCs, AT-MSCs	BM- and AT-MSCs attach to acellular human lung scaffolds	[37]
2014	Normal	Thin slices	MRC-5	rhCHI3L1 induces myofibroblast differentiation on acellular normal lung scaffolds	[19]
2014	Normal/IPF	Thin slices	Normal and IPF-derived HLFs	ECM contributed more significantly to IPF phenotype rather than cell origin	[38]
2014	Normal	Physiological instillation and thin slices	BM-MSCs, HBE, CBF12, HLFs	Segments of acellular lung scaffolds can be physiologically recellularised in three dimensions	[33]
2014	Normal/COPD	Physiological instillation, thin slices, aECM coating	BM-MSCs, HBE, CBF12, HLFs	Acellular scaffolds derived from COPD patients do not support viability comparable to those from normal lungs	[16]
2015	Normal	Whole lobe re-endothelialisation	HUVECs, BM-MSCs, iPSC-derived endothelial cells	Improved cell delivery techniques to vasculature and maintenance of cell viability; establishment of perfusable vascular lumens	[39]
2016	Normal	Thin slices	hVera101	Cells seeded onto acellular human lung scaffolds have less NF-κB and VCAM expression than when seeded onto acellular porcine lung scaffolds	[8]
2016	Normal/ scleroderma	Thin slices	PBMCs	Netrin-1 regulates fibrocyte transition on acellular lung scaffolds	[18]
2016	Normal	Whole lobe re-epithelialisation	HBE	HBE cells can be expanded and cultured *ex vivo* in whole acellular lung lobes	[40]
2016	Normal	Thin slices	Human islets	Acellular lung scaffolds support human islet function	[41]
2017	Normal	Thin slices	A549	CSE induces loss of epithelial cell markers; CSE treatment of cells on acellular lungs decreases proliferation as compared to standard tissue culture	[42]
2017	Normal (adult/ neonatal)	aECM coating	HBE	Neonatal lung aECM enhances proliferation; FBN-2 and TN-C increase proliferation	[31]
2017	Normal (adult/ paediatric)	Whole paediatric lung	AECs, HBE, endothelial cells, HUVECs	Multiple cell instillations with cell attachment and survival in whole lungs	[10]
2017	Normal/IPF	Thin slices	HLFs	HLFs did not adhere to IPF lung slices to the same extent as to normal lung scaffolds	[43]

Continued

TABLE 1 Continued

Year	Human lung status	Recellularisation technique	Cell type(s)	Significant finding	[Ref.]
2018	Normal/COPD	Floating bronchial rings	HBE	Bronchial ECM from COPD patients induces differential gene expression in primary HBE cells	[26]
2018	Normal/IPF	Thin slices	SAECs	Acellular scaffolds derived from IPF lungs induce loss of the epithelial phenotype	[15]
2018	Normal/IPF	Thin slices	PCs	Isolated PCs cultured on decellularised IPF lung matrices adopt expression of α-SMA	[17]

rATII: rat alveolar type II cells; IPF: idiopathic pulmonary fibrosis; HLF: human lung fibroblast; α-SMA: α-smooth muscle actin; iPSC: human induced pluripotent stem cell; ATII: alveolar type II; ATI: alveolar type I; MRC-5: Medical Research Council cell strain 5, human fibroblast cell line; SAEC: human small airway epithelial cell; mESC: murine embryonic stem cell; HFLC: human fetal lung cell; pBM-MSC: porcine bone marrow-derived mesenchymal stem cell; AEC: human alveolar epithelial cell; HUVEC: human umbilical vein endothelial cell; PAEC: pulmonary alveolar epithelial cell; NKX2-1: NK2 homeobox 1; ECM: extracellular matrix; BM-MSC: human bone marrow-derived mesenchymal stem cell; AT-MSC: human adipose tissue-derived mesenchymal stem cell; rhCHI3L1: recombinant human chitinase 3-like 1; HBE: human bronchial epithelial; CBF12: human endothelial progenitor cells; COPD: chronic obstructive pulmonary disease; aECM: ECM coatings for tissue culture plastic derived from acellular lungs; hVera101: human VeraVec cells, a modified umbilical cord endothelial cell line; VCAM: vascular cell adhesion molecule; PBMC: peripheral blood mononuclear cell; CSE: cigarette smoke extract; FBN-2: fibrillin-2; TN-C: tenascin-C; PC: human microvascular pericyte.

interactions in a high-throughput manner. To date, pepsin digestion and acid extraction have been used to obtain ECM solutions that can be used to coat tissue culture wells or polyacrylamide hydrogels of different stiffness [16, 17, 31]. These techniques have already been used to study a variety of cellular behaviours, such as cell adhesion to ECM components, cell viability, proliferation and cell migration, and to decouple mechanical changes in the ECM from its composition.

Use of acellular human scaffolds to study repair and disease

In parallel to the advances made for regenerating lung tissue *ex vivo* using acellular scaffolds, methods have been developed to generate acellular scaffolds derived from patients with chronic lung disease (table 1) [6, 16–18]. These techniques have opened new doors for studying cell–ECM interactions and have provided new insights into potential disease pathomechanisms, especially for chronic diseases where *in vivo* animal models do not adequately recapitulate human disease. Both disease and the age of the patient from which the acellular lung scaffold originates have been shown to affect initial cell adhesion, proliferation and survival of seeded cells [16, 31, 43].

In addition to histological characterisation using standard techniques, several groups have used techniques such as second harmonic generation and mass spectrometry based proteomics to characterise acellular scaffolds derived from chronic lung disease patients [6, 16, 18, 43]. In general, ECM components are difficult to detect using mass spectrometry based proteomics, owing to the dramatic differences in protein solubility between cell-associated proteins and ECM-associated proteins. Decellularisation [44] and other detergent solubilisation techniques that mimic decellularisation [45] have opened up new possibilities for examining differences in human lung ECM using mass spectrometry. Several studies have examined differences in the ECM derived from patients with chronic lung diseases, such as idiopathic pulmonary fibrosis [6] and emphysema [16], as well as from patients of different ages (neonatal lungs *versus* adult lungs) [31]. These studies have yielded important insights into differences in individual components within the ECM, such as major collagens, laminins and glycoproteins, which may be driving the phenotypic differences observed when cells are cultured on these scaffolds.

Protocols for decellularising whole human lungs or individual lobes may need to be adapted depending on the disease status of the patient. While protocols to decellularise individual lobes from normal patients could be used for patients with emphysema [16], these same protocols were found to be inadequate for patients with ILD [9, 15]. ILD causes altered tissue-level mechanics and thus the flow of decellularisation reagents throughout the tissue is altered when perfusion based decellularisation is used. Furthermore, even if segments are resected from these lungs, the parenchyma is extensively remodelled in ILD, resulting in heterogeneous regions of dense ECM. Thus, detergents may need to be used at higher concentrations or for longer periods of time to ensure adequate decellularisation compared to tissue derived from normal lungs [15].

Differences observed using the same decellularisation protocol in normal and diseased human lungs may be a result of tissue-level changes in disease but may also reflect differences in extractability of different

ECM components in disease, which could be due to changes in crosslinking frequency or disease-related degradation or secretion [46]. Due to the inherent heterogeneity of human samples, it is important that enough patients are included to overcome this variability and yield meaningful results. Furthermore, as multiple investigators, both in the lung field and in other tissues, have identified a large variety of non-ECM proteins in acellular human and animal scaffolds [9, 44], it is important for investigators using these *ex vivo* models to understand the limitations of using acellular scaffolds to study cell–ECM interactions. The effect of residual proteins, such as growth factors and other cell-associated proteins such as histones and cytoskeletal proteins, may not be negligible in these settings and may induce responses that do not correspond to *in vivo* environments [47]. Thus, while acellular human lung scaffolds are a powerful *ex vivo* system for studying cell behaviour in a three-dimensional environment and in many ways better reflect clinical heterogeneity and human disease compared to using animals that are genetically similar, it is important that the aforementioned limitations are taken into account when designing experiments and interpreting results.

Bioengineering: recellularisation of whole lung scaffolds

The bigger goal of utilising native ECM scaffolds to regenerate functional lung tissue still faces many questions and challenges. Successful recellularisation of these scaffolds will require the isolation and expansion of the necessary cell populations for tissue regeneration. Two main cell sources currently being investigated are 1) primary tissue-isolated progenitor cells [40] and 2) differentiated pluripotent stem cells [39, 48–50].

Primary cells provide a useful tool to study and develop effective lung recellularisation strategies. Cell lines including human umbilical vein endothelial cells and A549 alveolar epithelial cells have been used in numerous experiments to demonstrate biocompatibility to the decellularised scaffold. While useful, these cells possess less translational potential as they are not patient specific and may not be derived from normal tissues. Lung tissue-isolated cell populations have also been used in recellularisation studies. As a tissue that is under constant assault from the external environment, the lung possesses several endogenous progenitor cell populations that can be activated to regenerate tissue following injury or damage [51]. Emerging research has demonstrated that some of these progenitor cells may be capable of multi-lineage differentiation, contributing to both proximal and distal lung regeneration [52, 53], which could be exploited in complex lung tissue recellularisation. However, isolation and expansion of these rare cell types in sufficient numbers remains a barrier to their utility in whole organ recellularisation. Also, lung diseases underlying the need for transplantation will probably limit the function and expansion capacity of these primary cells. The use of induced pluripotent stem cell (iPSC)-derived cell populations has the potential to overcome the need for primary lung tissue. A terminally differentiated cell of any origin could, in theory, be reprogrammed to the pluripotent state and subsequently differentiated to numerous lung-specific cell types. Challenges in the efficiency, specificity and scalability of iPSC-differentiation protocols remain, and the issue of inherent diseases may still need to be solved. Donor-specific genetic diseases, such as cystic fibrosis, may have the possibility to be corrected in the iPSC state, prior to differentiation, although maintaining the genome integrity of the iPSCs and the differentiated cells is still an uncertainty [54]. The idea of a universal donor tissue or bank of tissues, from which iPSCs can be generated and differentiated to match donors regardless of tissue type, is an exciting potential solution to this problem [55], but remains under development.

Even with adequate numbers of the correct cell populations, efficient repopulation of the scaffolds in a site-specific manner poses another challenge. This will require methods to organise and pattern the redelivered cells into the three-dimensional structure of the acellular scaffold. Building upon the native structure, mature cells will either need to be directed to the correct anatomical space (*e.g.* proximal *versus* distal lung epithelium) or be delivered in a progenitor state and targeted to specifically differentiate once in the lung scaffold. The microenvironment, including growth factors, local oxygen tension, cell–cell interactions and dynamic tissue mechanics, can be manipulated to direct higher-level tissue organisation [56, 57]. This process will require the design of specific bioreactors to provide the optimal regenerative environment for *ex vivo* tissue maturation. For lung bioengineering, this includes vascular perfusion, the capacity for fluid or air ventilation, and the ability to test functional end-points of tissue regeneration, including gas exchange, cellular metabolism and barrier function [58]. The ability to recapitulate key developmental cues to the regenerating organ is a main advantage of custom-designed bioreactors, which will aid in the creation of optimised and targeted organ culture protocols that drive innate repair mechanisms.

Translation to clinically relevant, transplantable grafts

A major aim of the field is to create therapeutic solutions for end-organ failure and alleviate the current donor shortage, making lung transplantation a patient-specific, on-demand process. Progress toward this

goal has utilised porcine scaffolds, recellularised with human cell populations, followed by *ex vivo* culture and re-implantation to porcine recipients [2, 59]. Decellularised porcine scaffolds were repopulated with human umbilical vein endothelial cells in the vascular network and basal epithelial stem cells in the airways. Following 6 days of *ex vivo* culture, the constructs were transplanted, and vascular reperfusion and ventilation re-established, for 1 h of functional assessment. Basal gas exchange was achieved, although an immature barrier function and lack of pulmonary surfactant limited overall function of the graft. Challenges in vascular perfusability and coagulation also remain in the path towards the goal of successfully engineering a graft with long-term survival. Important questions regarding the necessity of other cell types, as well as the innate and acquired immune system response to engineered tissues based on either human or xenogeneic scaffolds, must be addressed once extended graft survival is achieved [60]. As advances in bioengineering methods are achieved and graft function improves, extensive preclinical testing in large animal models will be an important requirement towards human transplantation. Due to the potential for large patient-to-patient heterogeneity and complexity in translational parameters (*e.g.* scaffold source, cell source and *ex vivo* culturing parameters), sufficient numbers of studies will be required to thoroughly validate any clinical-grade procedure and effectively translate this technology to a therapy.

Conclusion

The creation and use of acellular lung scaffolds has provided important new tools and opportunities for studying lung repair and regeneration, and furthermore provides a more physiological setting for studying human lung diseases. If the goal of utilising *ex vivo* lung bioengineering approaches to create functional, transplantable grafts can be realised, this approach has the potential to overcome the challenges of organ donor shortages and prevent allograft immune rejection. Ultimately, progress in the fields of developmental biology, cell biology, physiology and transplantation can be combined to create therapeutic solutions for lung disease and end-organ failure.

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