How to build a lung: latest advances and emerging themes in lung bioengineering

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ABSTRACT Chronic respiratory diseases remain a major cause of morbidity and mortality worldwide. The only option at end-stage disease is lung transplantation, but there are not enough donor lungs to meet clinical demand. Alternative options to increase tissue availability for lung transplantation are urgently required to close the gap on this unmet clinical need. A growing number of tissue engineering approaches are exploring the potential to generate lung tissue *ex vivo* for transplantation. Both biologically derived and manufactured scaffolds seeded with cells and grown *ex vivo* have been explored in pre-clinical studies, with the eventual goal of generating functional pulmonary tissue for transplantation. Recently, there have been significant efforts to scale-up cell culture methods to generate adequate cell numbers for human-scale bioengineering approaches. Concomitantly, there have been exciting efforts in designing bioreactors that allow for appropriate cell seeding and development of functional lung tissue over time. This review aims to present the current state-of-the-art progress for each of these areas and to discuss promising new ideas within the field of lung bioengineering.

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Introduction

Background

Respiratory diseases are the third leading cause of death worldwide and are predicted to continue to increase over the coming years. The overall cost of respiratory disease in the European Union amounts to more than EUR380 billion annually [1]. Currently, the only option for end-stage respiratory disease is lung transplantation. Approximately 2000 lung transplants occur annually in Europe [2], with the same number or more patients awaiting transplantation. Transplant efficacy remains a significant clinical issue as transplant rejection rates are high and complications can arise due to the required immunosuppressive drugs; 5-year survival is only 50% [3]. Alternative options to increase available tissue for lung transplantation are necessary to close the gap on this unmet clinical need. In addition to the use of lungs donated from circulatory death (DCD) donors, and continued development and improvements in *ex vivo* preservation and *ex vivo* lung perfusion (EVLP) techniques attempting to maximise the number of donor lungs suitable for transplantation [4–9], an exciting new area of research focuses on generating lung tissue *ex vivo*.

Novel ideas and hypothesis

The lung is a highly complex and dynamic organ comprised of a number of different cell types with distinct functions [10]. Bioengineering lung tissue *ex vivo* for transplantation is an area receiving increased attention and could address the shortage of donor organs. Current approaches being explored in pre-clinical studies utilise biologically derived or synthetic scaffolds that are seeded with autologous cells from the eventual transplant recipient. Both synthetic and biologically derived scaffolds have distinct advantages and disadvantages. Hybrid scaffolds combining biologically derived and synthetic scaffolds may be a novel approach to limit the disadvantages observed with either synthetic or biologically derived scaffolds alone. Various different technologies have been developed to help generate tissue engineering scaffolds for lung, such as decellularisation for biological scaffolds, and advanced manufacturing processes for producing synthetic scaffolds, such as casting, electrospinning, cryogelation and microfabrication techniques.

Thus, bioengineered lung tissue could help to increase the amount of lung tissue available for transplantation and has the potential to offer benefits over allogeneic transplantation. In this review, we aim to discuss recent advances and emerging themes in lung tissue engineering, and the major challenges that need to be overcome to advance this approach closer to the clinic.

Manufacturing lung scaffolds

The lung has at least 40 different resident cell types [10], all of which are necessary for optimal functioning. These cells reside on and within an extracellular matrix (ECM) comprised of different regional combinations of ECM proteins and glycosaminoglycans (*e.g.* proteoglycans and hyaluronan) that act together as a scaffold to not only provide structure, but also to help direct repair and regeneration following injury [11]. Langer and Vacanti [12] first described tissue engineering approaches, whereby cells are combined with a matrix made of natural or synthetic materials and grown *ex vivo*, followed by transplantation (figure 1). Several case reports and clinical trials of tissue-engineered products have since demonstrated the feasibility of pursuing these technologies in the clinic [13, 14]. Although there were no attempts at generating pulmonary tissue *ex vivo* when tissue engineering was first described, most current approaches have adopted this paradigm. Despite the fact that lung tissue engineering approaches have historically lagged behind those of other fields, there have been exciting recent advances with both natural materials and synthetic materials.

Acellular lung scaffolds

Scaffold source and effects of processing, storage and sterilisation

Acellular (biological) lung scaffolds have emerged as possible scaffold materials for *ex vivo* lung tissue engineering in recent years. In this approach, acellular scaffolds are obtained by removing the cells from native lung tissue *via* a method called decellularisation. Ultimately, the goal of any decellularisation protocol is to remove the cellular material without adversely affecting the resulting macroscopic acellular scaffold structure and ECM composition, mechanical integrity or biological activity of the ECM components [15–17]. The acellular lung could then ideally be recellularised with autologous cells or, alternatively, an allogeneic source. A major advantage of acellular lung scaffolds is that they mostly retain the complex structure and macro- and micro-architecture of the native lung tissue, which cannot be generated using any known manufacturing techniques. The majority of ECM components have been found to be retained in the acellular tissue following decellularisation [18–20], although the degree to which these components are retained in their native orientation has not been studied extensively. The retention of ECM components, as well as their organisation, is probably vital for the function of the eventual engineered tissue.

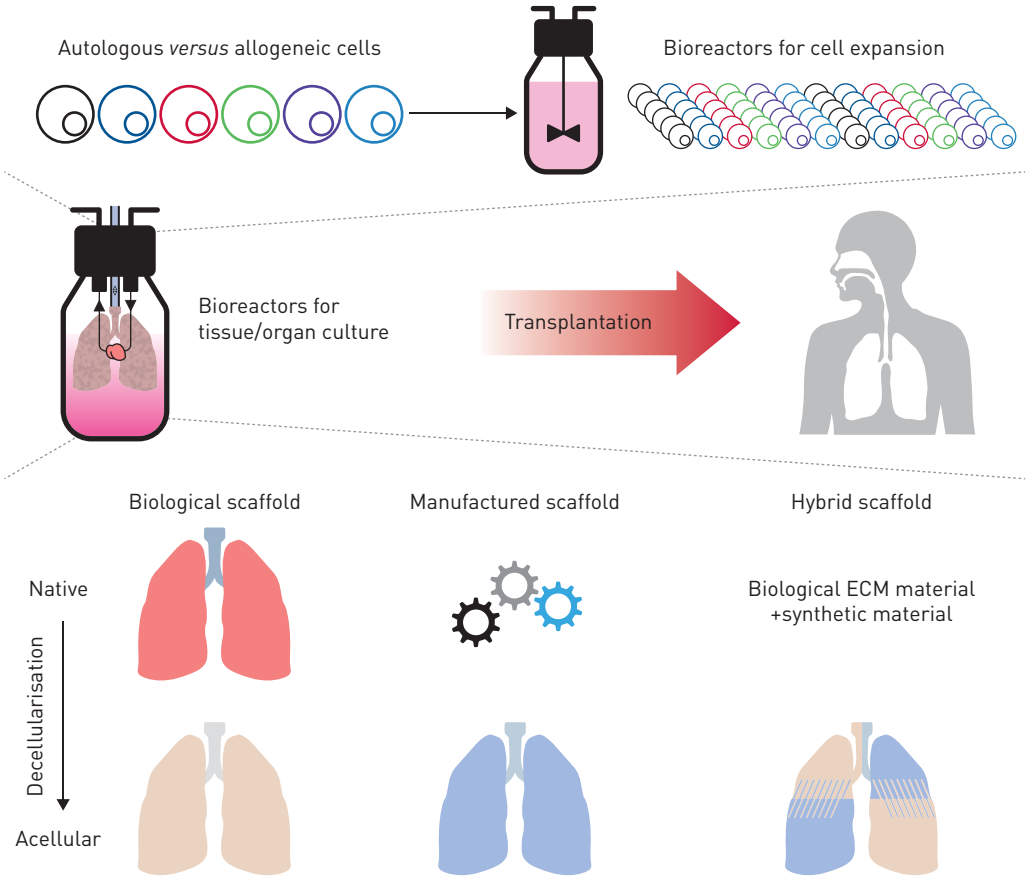

FIGURE 1 Lung bioengineering approaches. In most approaches, a lung scaffold is seeded with autologous or allogeneic cells for bioengineering a lung. The cells can be expanded to appropriate numbers in bioreactors for cell expansion. Different lung scaffolds have been explored, including decellularised scaffolds and synthetic scaffolds. An emerging idea is the use of hybrid scaffolds that combine biological materials such as extracellular matrix (ECM) components with synthetic scaffolds in order to create a hybrid lung scaffold. Bioreactors for organ culture can then be used to mature and evaluate the repopulated lung scaffold before lung transplantation.

The ECM is one of the major constituents of the microenvironment known to direct cell behaviour such as migration, proliferation and differentiation [18, 21, 22]. Acellular tissue scaffolds have been shown to retain bioactive properties and a certain degree of tissue specificity [19, 23]. Recent studies have shown that aged scaffolds and those derived from lung disease can drive the acquisition of deranged cellular phenotypes in cells from normal patients [21, 24–27]. Thus, human scaffolds from aged patients or those with pre-existing lung diseases are likely not the ideal sources for lung tissue engineering scaffolds.

Interestingly, a recent study indicated that scaffolds derived from early post-natal human lungs support enhanced re-epithelialisation compared with those derived from adult lungs [28]. Although post-natal lungs could be used to generate scaffolds for neonates, a major concern with using neonate lungs for adult lung tissue engineering is the size mismatch. A study that investigated the outcome of lung transplantation from size-mismatched donors and recipients found that undersized lungs received higher tidal volumes because of differences between the weight of donors and recipients [29, 30]. Additionally, lung transplants using undersized lungs are associated with an increased risk of primary graft dysfunction [31]. Although it is unclear how or if lungs from neonates might be used for adult lung tissue engineering schemes, the information gained from these studies may lead to new ideas for utilising scaffolds derived from adult donors.

When considering sources for clinical-grade scaffold materials, the donor tissue does not necessarily need to be of human origin. Lungs from anatomically similar species, such as porcine or nonhuman primates, may potentially provide a more uniform donor source, with less limitations than human sources [19, 25, 32–35]. The use of nonhuman primate lungs has been restricted to pre-clinical models of bioengineering and there are ethical concerns with using nonhuman primates as a source of acellular scaffolds. Thus, porcine lungs have first emerged as a potential option due to the use of other porcine tissues in

xenotransplantation. However, several species- and tissue-specific properties have been identified to date in porcine lungs that may prove challenging for translation of xenogeneic lung scaffolds recellularised with human cells. Pleural blebs (cystic spaces) have been shown by us and others to arise during the decellularisation process, which may affect the ventilation mechanics of the lung, ultimately compromising the function of the lung if transplanted [24, 33] and if ruptured would lead to pneumothorax (collapsed lung). Furthermore, the extent to which porcine lungs have collateral ventilation is limited compared with humans. Although a variety of cell types have been shown to adhere to porcine scaffolds [24, 25, 33, 35], human-derived endothelial cells were found to attach to porcine-derived scaffolds at a lower rate compared with human- or primate-derived scaffolds [36], but the reasons for this remain unknown. Residual, cell-associated xenogeneic proteins known to cause negative immunogenic responses in humans have been identified in scaffolds from porcine lungs that are decellularised according to current criteria [18, 25], thus indicating that removal of immunogenic proteins either through transgenic approaches or through post-decellularisation treatments might be necessary.

Moreover, there are several studies from human cohorts of transplantation that indicate the importance of size matching between donor and recipient [37]. In the case of porcine lungs, the airways and vasculature are often smaller than in human lungs. Surgically anastomosing a porcine lung to a human recipient, especially with regard to the bronchus, will be challenging and a donor–recipient mismatch is likely to occur that can lead to death. The lower pulmonary lobes of porcine lungs are shaped after the pig's body because it is a quadruped. The lower pulmonary lobes have a pointed shape and the lung ligaments are significantly more pronounced than in humans. Therefore, basal atelectasis of the lower lobes of the lung may occur if the lung is transplanted to a human and hence there is an increased risk of infection. The lower pulmonary lobes could be resected at the time of transplantation, but it is unclear if this would be optimal. Some transplant centres carry out lung resections at the time of transplantation to make the lung fit better into the thoracic cavity. However, some transplant centres are negative towards this approach because of the risk of air leakage from the lung post-operatively. In addition to the major anatomical differences, the risk of cross-species transmission of porcine endogenous retroviruses has impeded the clinical application of xenogeneic tissues [38]. To date, there has been no formal study of the removal or retention of zoonoses, including porcine endogenous retroviruses, in xenogeneic decellularised scaffolds and thus the danger of disease transmission across species remains unknown [36]. Therefore, adult human lungs that narrowly miss the criteria for transplantation and cannot be improved using EVLP technology [6, 7, 39–41] may be viewed as the most likely candidates as a source of scaffolds for a clinical-grade lung tissue engineering scheme.

Storage and sterilisation of potential acellular scaffolds for lung tissue engineering have been found to significantly impact both the structure and residual protein content. Moreover, the ability of different cell types to survive and proliferate following inoculation has also been shown to be influenced by the conditions of storage and sterilisation of acellular scaffolds [42]. To date, the majority of acellular lungs have been sterilised using peracetic acid; however, this can result in ECM degradation [43, 44]. A recent report described the use of supercritical carbon dioxide for sterilising acellular lung scaffolds that does not induce degradation of the ECM [45]. Excellent progress has been made in techniques that could be compatible with Good Manufacturing Practice (GMP) [46]. Interestingly, despite the establishment of some baseline criteria, commercially available scaffolds for other tissues from different companies can have different responses in the same model of *in vivo* injury [47]. It is clear that defining optimal criteria and end-points regarding the scaffold source and processing of acellular scaffolds will be important in future studies (see table 1 for a summary of the challenges within the field of *ex vivo* lung tissue engineering).

Decellularisation techniques

Common methods to decellularise lungs include different combinations of physical, ionic, chemical and enzymatic methods [15]. Detergent-based perfusion has been most prevalently utilised to generate acellular lung scaffolds. Commonly used detergents include Triton X-100, sodium deoxycholate, sodium dodecylsulfate and CHAPS (3-((3-cholamidopropyl)dimethylammonio)-1-propanesulfonate), which are used with or without hypertonic sodium chloride and DNase and/or RNase solutions. The concentration and volume of detergents used varies between different protocols and species [21, 23, 24, 26, 33, 34, 36, 48–51]. Solutions may be perfused through the vasculature or both the airways and vasculature. The variation between different protocols has resulted in apparent histological differences of the decellularised lungs and in the content of both ECM and other retained proteins [50, 52, 53]. It remains unclear how differences in lung decellularisation protocols might affect recellularisation and regeneration or potential immunogenicity of the implanted scaffold [54, 55]. The majority of laboratories decellularising tissue utilise the criteria set forth by Crapo *et al.* [18], which include absence of visible cellular or nuclear material on histological examination, <50 ng double-stranded DNA per 1 mg of dry

TABLE 1 Challenges within the field of *ex vivo* lung tissue engineering

Area	Focus for future research/future perspectives
Scaffold source	Can a suitable acellular xenograft source be identified? Can allogeneic human scaffolds be used? Does the age (neonatal or aged) of the scaffold impact the biomaterial? Evaluate immunogenicity of scaffold with and without cells.
Cell sources	Are all of the more than 40 cell types found within the lung required to make a functional lung? Can allogeneic cells be used or do we need to use autologous cells? Where will we source cells for patients with chronic or genetic lung diseases? What types of cell sources can be used (*e.g.* endogenous progenitor cells, induced pluripotent stem cells)?
Manufacturing	Which Good Manufacturing Practice manufacturing method will be suitable for scaffold generation, storage and maturation? Which standardised approaches for the characterisation and validation of the scaffold will be required? How can we obtain enough cells to recellularise and how will they be re-introduced into the scaffold? Will bioengineered lungs need to be tailored for patients with specific lung diseases (*e.g.* the main lung transplant recipients (chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, pulmonary arterial hypertension, cystic fibrosis and α_1-antitrypsin deficiency)).
Maturation	Will different bioreactors be needed for the different cell types in the lung? What time span and/or maturation level will be required? What degree of vascularisation of the scaffold will be required?
Surgical and clinical approach	How will we assess the functionality prior to transplantation? Will *ex vivo* lung perfusion parameters be enough to predict success? What surgical techniques could be used for pieces of bioengineered lung tissue? Will special post-operative care be required? Will the patients need to be immunosuppressed?

weight of the ECM scaffold and remnant DNA <200 bp. However, these are minimum criteria that do not take into account cytocompatibility (*e.g.* effects of residual decellularising agents), sterility, composition and mechanical properties of the acellular scaffold [56, 57]. ECM composition and mechanical properties are regionally specific, and retention of these differences may be important for recellularisation leading to functional tissue.

Recellularisation and pre-clinical transplantation models

A variety of different cell types have been successfully used in recellularisation of acellular scaffolds, including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) and endogenous lung progenitor cells. Efficient differentiation of pluripotent cells to proximal and distal lung epithelial cells remains a challenging task, but there has been exciting recent progress in deriving distal epithelial progenitor cells and multilayered epithelium from iPSCs and murine ESCs [50, 51, 58–65].

Differentiation of pluripotent stem cells into proximal or distal lung epithelial cells requires growth factors known to be sequestered by the lung ECM [66]. Thus, retention of ECM components and their associated growth factors in decellularised lung scaffolds may be critical for optimal recellularisation with regional specificity. The ability of the reseeded cells to survive, proliferate and differentiate is important for assessing both short- and long-term cytocompatibility of the scaffold. Further understanding of how the remaining ECM and residual protein composition may affect seeded cell types over time will be an important area of future studies (table 1).

Important proof-of-concept studies have shown that acellular lung scaffolds can be recellularised with either single-cell suspensions from fetal lung homogenates and re-endothelialised with immortalised endothelial cell lines or iPSC-derived endothelium and epithelium [67] for transplantation. When transplanted into rats, recellularised lung scaffolds were shown to briefly function *in vivo* [48, 51, 68, 69] and, more recently, acellular porcine scaffolds recellularised with human cells in scaled-up porcine studies [35]. However, transplanted lungs were oedematous with regions of collapsed architecture, indicating that long-term functional lung regeneration has yet to be achieved. Nonetheless, these studies encourage the feasibility of this approach.

The Goldilocks Principle of the remaining proteins: "too much", "too little" and finding "just right"

The purpose of most decellularisation processes is to remove the cellular and immunogenic material from the scaffold, while retaining the ECM proteins and structure. However, as mass spectrometry proteomics have rapidly improved to characterise the composition of acellular scaffolds, it has become increasingly evident that large amounts of non-matrisome proteins (cytoskeletal elements and cell-associated proteins) are retained in the scaffold following decellularisation [21, 24]. The presence of non-matrisome proteins has been observed across different tissues, species and techniques used in different laboratories [50, 64, 70]. The impact of these residual proteins and other cell-derived components on reseeded cells and their potential immunogenicity have been relatively unexplored for lung tissue engineering. It remains unclear if 1) decellularisation protocols that are more aggressive and remove more components or 2) protocols that are less aggressive but retain more components will be more beneficial in supporting functional regeneration. Residual proteins have been shown to play a significant role in other tissues in regulating cell behaviour of reseeded cells and on immune cell infiltrates once implanted [71]. Furthermore, the clinical heterogeneity observed between individual healthy patients may make defining minimum criteria challenging [24]. Ultimately, improving our understanding of the composition of decellularised scaffolds and tying these to biological outcomes is an important future direction for the field.

Table 2 and the following sections summarise some of the most important studies to date that have led to advances inusing acellular scaffolds for lung tissue engineering [22, 24, 25, 33, 34, 42, 48, 49, 51, 52, 59, 64, 65, 72–128].

Artificial lung scaffolds

Although acellular scaffolds show promise in the field of lung bioengineering, the heterogeneity of human lung-derived acellular scaffolds and potential xenogeneic issues make this approach challenging to scale-up in a reproducible and controllable manner [21]. An alternative to acellular scaffolds could be artificial (or manufactured) scaffolds. Both synthetic and natural polymers can be used in these approaches, and a variety of materials have already been explored for tracheal, bronchial and parenchymal lung tissue engineering (figure 2 and table 2). Artificial scaffolds for tracheas have been more intensively investigated due to the trachea's simpler, tubular structure [72–78]. Many of the materials used to generate scaffolds for pre-clinical studies for large airways are synthetic polymers such as POSS-PCU (polyhedral oligomeric silsesquioxane poly(carbonate-urea) urethane) [72], polyglycolic acid, pluronic F-127 [79] and polylactic-*co*-glycolic acid [80], which are all cytocompatible polymers with mechanical properties in the range needed for tracheal tissue engineering. Although these materials can be manufactured with good precision and can be processed to have improved storage stability, most lack the necessary biological properties, such as native integrin binding sites and bioactive cues for cellular attachment, proliferation and differentiation. It is currently unknown what motifs will need to be added to these scaffolds to support functional regeneration. However, it has been shown that cell seeding and graft coverage can be enhanced by simply modifying the surface of the polymer used in the scaffold, *e.g.* providing cell attachment sites by increasing surface porosity [72] and/or incorporating individual ECM components [73].

On the contrary, parenchymal lung tissue has a more complex three-dimensional (3D) structure, and requires thin boundaries and interconnected pores for efficient gas exchange [81]. Due to the difficulty associated with manufacturing a scaffold with geometrical parameters suitable for parenchymal lung tissue, there have been limited reports. To date, potential scaffolds for parenchymal tissue engineering have been fabricated *via* foaming [82, 83], porogen-solvent techniques, cryogelation [84], photodegradation [85, 86] and self-assembly of microspheres [87]. Although these techniques are able to recapitulate alveolar-like structures, they lack the vasculature and airways required for integration into recipients, and ultimately for gas exchange. However, a recent report showed that a 3D gelatine microbubble scaffold seeded with murine pluripotent stem cells promoted angiogenesis when implanted [82]. Thus, if these constructs could somehow be hooked up to an air supply and the boundaries of the blood–air barrier were thin enough, it is conceivable that these constructs could support gas exchange. Although important as proof-of-concept studies, none of these subtractive or bulk manufacturing techniques have been successful in generating functional lung tissue and incorporation of vasculature has not yet been explored. Synthetic materials could be of immense value for generating lung scaffolds due to the ability to precisely and reproducibly manufacture them for individual patients, but suitable manufacturing methods are not yet known. However, there are approaches that have been used in other organs that may be worthwhile exploring for lung tissue which will be discussed in the following section.

Potential manufacturing methods to generate porous scaffolds for lung tissue engineering

From a simplistic viewpoint, the lung parenchyma is a system of interconnected porous-like structures surrounded by a capillary bed to facilitate gas exchange [81]. There are several different

TABLE 2 Compilation of studies of breakthrough advances within the field of *ex vivo* lung tissue engineering

Year	Material	Method	Scaffold	Significant advance	End-points	Reference
Synthetic						
2006	Polyglycolic acid and pluronic F-127 hydrogel	Microfabrication techniques	Alveoli-like structures	Growth of lung progenitor cells on a synthetic scaffold and transplanted	*In vitro*, *in vivo*	[79]
2006	Poly-DL-lactic acid	Microfilm templates and 3D foam	Alveoli-like structures	Alveolar epithelial cells can be grown on porous synthetic materials	*In vitro*	[120]
2012	Decorin-containing matrices	Electrospinning	Trachea	Electrospinning decorin matrices for a tissue-engineered trachea	*In vitro*	[73]
2013	Hydroxyethyl methacrylate–alginate–gelatine cryogel	Cryogelation	Alveoli-like structures	Macroporous matrix with ability to recruit cells when implanted *in vivo*	*In vitro*, *in vivo*	[84]
2014	Gelatine/microbubble scaffold	Microfluidics	Alveoli-like structures	Differentiation of lung stem/progenitor cells into alveolar pneumocytes and induction of angiogenesis within a manufactured scaffold	*In vitro*, *in vivo*	[82]
2015	Polyethylene glycol-based hydrogel	Microsphere templates	Alveoli-like structures	Cytocompatible manufacturing method for co-culture of alveoli-like structures	*In vitro*	[86]
2016	POSS-PCU (polyhedral oligomeric silsesquioxane poly (carbonate-urea) urethane)	Dispersion of porogens	Trachea	Use of engineered pores to improve integration capacity of a synthetic scaffold	*In vitro*, *in vivo*	[72]
2017	Alginate beads	Alginate beads	Alveoli-like structures	Self-assembled alveoli-like structures with human cells	*In vitro*	[87]
2018	Matrix metalloproteinase-degradable polyethylene glycol-based hydrogel	Microsphere templates	Alveoli-like structures	Controlled degradation with specific matrix metalloproteinase-cleavable sites in alveolar-like structures	*In vitro*	[85]
Acellular						
1981	Alveolar basement membrane (various origins)	Decellularisation	Alveolar basement membrane	First decellularisation attempt to obtain alveolar basement membrane	*In vitro*	[121]
1986	Human alveolar and amniotic matrix	Decellularisation	Acellular alveolar *versus* amniotic basement membranes	First repopulation experiment on acellular lung tissue; differentiation on various basement membranes	*In vitro*	[122]
2010	Rat lung	Decellularisation	Rat acellular lung	Orthotopic transplantation	*In vitro*, *in vivo*	[68]
2010	Rat lung	Decellularisation	Rat acellular lung	Orthotopic transplantation and first report of decellularisation of human lung	*In vitro*, *in vivo*	[48]
2011	Rat lung and liver	Decellularisation	Rat acellular lung and liver	Cellular differentiation on the scaffolds	*In vitro*	[123]
2012	Mouse lung	Decellularisation	Mouse acellular lung and slices	Comparison of different detergent-based protocols for mouse lung de- and recellularisation	*In vitro*	[52]
2012	Human lung	Decellularisation	Human acellular lung and slices	De- and recellularisation of human normal and fibrotic lungs	*In vitro*	[49]
2013	Human and porcine lung	Decellularisation	Human and porcine lung and slices	De- and recellularisation of human and porcine lungs	*In vitro*	[33]
2013	Mouse lung	Decellularisation	Mouse acellular lung and slices	Effects of age and emphysematous and fibrotic injury on murine recellularisation	*In vitro*	[124]

Continued

TABLE 2 Continued

Year	Material	Method	Scaffold	Significant advance	End-points	Reference
2013	Mouse lung	Decellularisation	Mouse acellular lung and slices	Effects of storage and sterilisation on de- and recellularised whole lung	*In vitro*	[42]
2014	Human and porcine lung	Decellularisation	3D lung segments	Small segments to retain 3D lung structure in acellular scaffolds from large animals and human origin for physiological recellularisation	*In vitro*	[24]
2014	Rat and human lung	Decellularisation	Rat and human acellular lung	Transplant of iPSC-derived re-epithelialised and re-endothelialised scaffold	*In vitro*, *in vivo*	[51]
2015	Rat and human lung	Decellularisation	Rat and human acellular lung	Regeneration of functional pulmonary vasculature	*In vitro*, *in vivo*	[65]
2016	Porcine lung	Decellularisation	Porcine lung extracellular matrix hydrogel	First extracellular matrix hydrogel derived from acellular lung	*In vitro*, *in vivo*	[125]
2016	Porcine lung: wild-type and α1,3-galactosyltransferase knockout	Decellularisation	Porcine lung	Comparison of de- and recellularisation of wild-type and α1,3-galactosyltransferase knockout pig lungs; identification of residual immunogens in wild-type lungs	*In vitro*	[25]
2017	Porcine lung	Decellularisation	Porcine lung	Orthotopic transplantation of porcine scaffold recellularised with human cells	*In vitro*, *in vivo*	[35]
Hybrid						
2006	Polylactic-*co*-glycolic acid, poly-L-lactic acid and Matrigel porous foam and nanofibrous matrix	Microfabrication techniques	Alveoli-like structures	First hybrid material attempt for lung tissue engineering	*In vitro*	[80]
2008	Matrigel plug combined with fibroblast growth factor 2-loaded polyvinyl sponge	Microfabrication techniques	Vascularised pulmonary tissue constructs	Distal pulmonary epithelial differentiation can be maintained *in vivo*; donor-derived endothelial cells contribute to the formation of vessels	*In vitro*, *in vivo*	[126]
2011	Lung extract-coated poly-ε-caprolactone nanofibres	Electrospinning	Electrospun nanofibres coated with lung extracts from fibrotic or nonfibrotic mice	*Ex vivo* system to recapitulate the 3D fibrotic lung microenvironment	*In vitro*	[22]
2011	Collagen–Matrigel/alginate microcapsules	Microsphere encapsulation	Alveoli-like structures	Fibroblasts, epithelial cells and alveolar type II form alveolus-like structures in collagen–Matrigel/alginate-poly-L-lysine-alginate microcapsule engineered scaffolds	*In vitro*	[127]
2017	Poly-ε-caprolactone and decellularised aorta	Electrospinning, decellularisation	Electrospun poly-ε-caprolactone stents in acellular rabbit aorta	Hybrid trachea scaffold for tracheal replacement	*In vitro*, *in vivo*	[128]
Cells						
2014	Human pluripotent stem cells	NA	NA	Functional human pluripotent stem cell-derived distal lung epithelial cells seeded onto human scaffold	*In vitro*, *in vivo*	[59]
2015	Human endothelial and perivascular cells	NA	NA	Regeneration of functional pulmonary vasculature	*In vitro*, *in vivo*	[65]
2016	Human respiratory epithelial cells	NA	NA	Scalable cell culture system	*In vitro*, *in vivo*	[107]

Continued

TABLE 2 Continued

Year	Material	Method	Scaffold	Significant advance	End-points	Reference
2016	$KRT5^+TP63^+$ basal epithelial stem cells	NA	NA	Recellularisation	*In vitro*	[64]
2018	Chondrocytes, endothelial cells and mesenchymal stem cells	3D bioprinting	None	Scaffold-free manufacturing of a rat trachea mimic	*In vitro*, *in vivo*	[101]

3D: three-dimensional; iPSC: induced pluripotent stem cell; NA: not applicable.

manufacturing methods that have been used for tissue engineering porous structures for other organs, *e.g.* freeze-drying, foaming, solvent-casting and particulate-leaching techniques [88]. Manufacturing processes have also been developed for generating perfusable vascular channels in tissue-engineered constructs [89].

Electrospinning is an additive manufacturing technique that has emerged as an effective method of producing nanoscale fibres for use in multiple fields, including tissue engineering of blood vessels [90], skin [91] and trachea [73]. Both synthetic and natural polymers can be used for electrospinning to create porous scaffolds comprised of thin nanofibres that have been shown to be capable of supporting cell attachment, proliferation and differentiation. By controlling fibre parameters such as size, density, composition and orientation, fibrous structures that are similar to the ECM can be produced. Moreover, molecules such as growth factors or pharmaceuticals can be included in the scaffold to influence and direct regeneration of the tissue spatially or temporally. Electrospinning has already been used as an *in vitro* platform for studying the effects of fibrotic lung micro-environments on various cell types [22]. Overall, electrospinning is a promising technique, but has been limited to only creating an *in vitro* assay platform and has not yet been used for bioengineering lung tissue.

Techniques that are amenable to creating custom-made, reproducible, intricate 3D designs using cytocompatible materials would be ideal for tissue engineering. 3D printing, or bioprinting when cells are printed, has recently emerged as a potential source for bioengineering tissues or supporting structures [92–98]. 3D printing has been applied clinically for the treatment of tracheobronchomalacia [99] and tracheal collapse with 3D printed patient-specific tracheal splints [100]. However, in these instances, the 3D printing technology was simply used for supporting structures and not for regenerating transplantable tissue in humans. 3D printing has been explored for tissue engineering of organs such as rat trachea using a scaffold-free approach [101], skin [102], cartilage [103], aortic valve conduits [104] and vascular tissue [105], but there are currently no published reports of attempts to 3D print lung tissue capable of gas exchange. 3D bioprinting of distal lung will be extremely challenging, as the gas exchange barrier is of the order of nanometres and nozzles used for printing cells need to be in the micrometre range. Thus, new 3D printing or manufacturing approaches need to be developed to overcome this limitation. However, 3D printing technologies are advanced enough for printing structures at the resolution of the trachea and bronchus (figure 2), but materials and manufacturing methods compatible with cells have not yet been reported.

One challenge in 3D printing of biological tissue is that many current techniques for printing high-resolution structures use processes that are incompatible with directly printing cells and many biological materials. Traditional 3D printing approaches rely on solvents or heating to generate polymer solutions that can flow as liquids through the 3D printing nozzles. Thus, these limitations have contributed to slow progress in 3D printing of lung and other tissues.

It is important that the material printed does not degrade before new ECM is synthesised, deposited and organised by seeded cells that can support the structure of the tissue and handle any mechanical loading (*e.g.* further cell seeding, bioreactor parameters and surgical handling) [106]. Thus, the degradation kinetics and the impact of the degradation products are important parameters to consider when selecting, designing and validating potential new biomaterials for pulmonary tissue engineering applications. These and other issues need to be addressed for successful application of manufactured scaffolds for generating lung or airway tissue.

Hybrid materials

Although both acellular scaffolds and manufactured scaffolds have shown progress, ultimately neither may be the optimal scaffold material alone. In many other manufacturing fields, optimal materials are derived through the use of hybrid or composite materials whereby the positive attributes of two or more materials can be combined to generate a final material with optimal overall properties that overcome the limitations of the individual constituent components. Using hybrid or composite materials to manufacture scaffolds

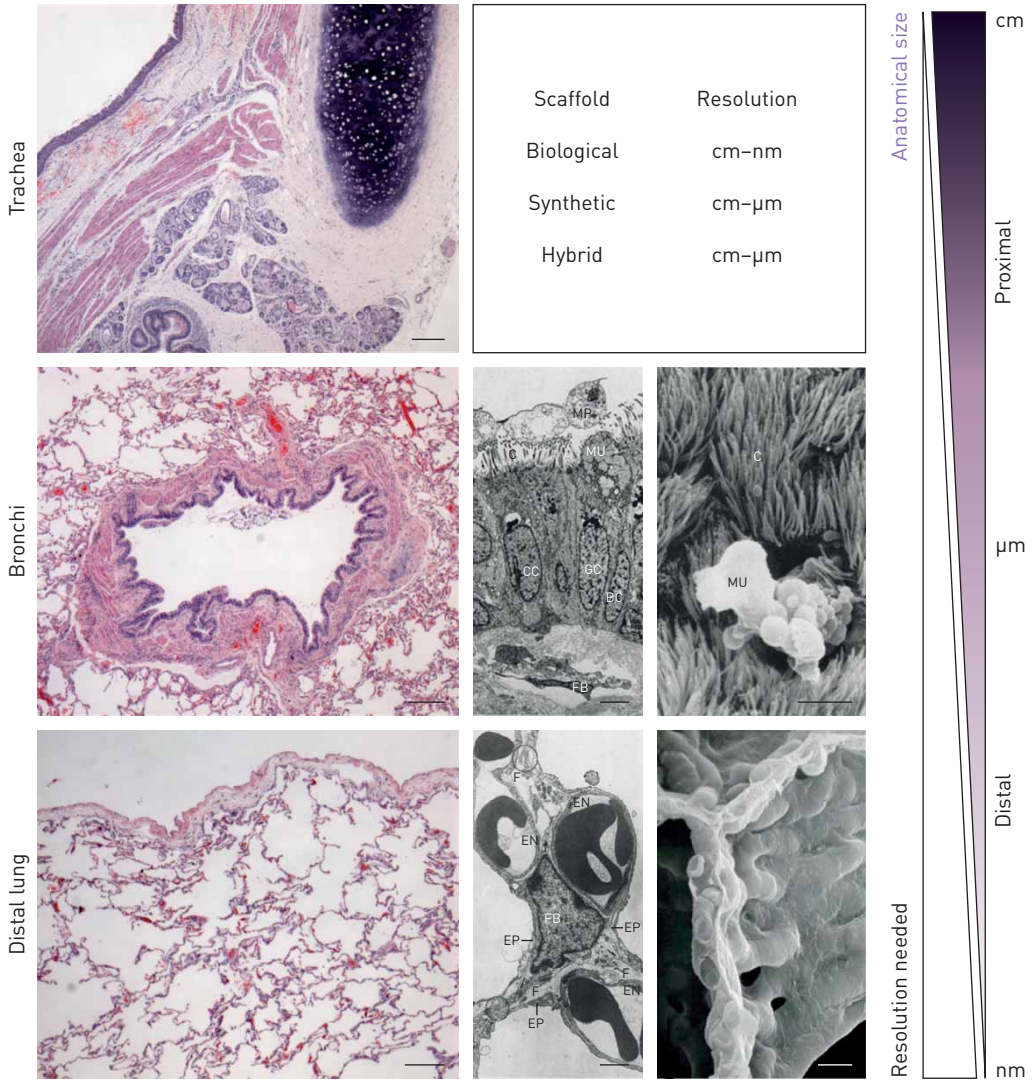

FIGURE 2 Recapitulating the complexity of the lung architecture from proximal (trachea) to distal (bronchi and alveoli): haematoxylin/eosin (HE; left column) staining histology, transmission electron microscopy (TEM; centre column) and scanning electron microscopy (SEM; right column) images from proximal and distal native lung tissue. The lung architecture varies dramatically within the lung: moving from proximal to distal, the resolution required to mimic the native structures is higher (centimetres (cm)→micrometres (μm)→nanometres (nm)). To date, biological scaffolds are the only scaffolds that have a resolution that mimics that of the native lung across all length scales. The bronchi TEM micrograph of a thin section of the mucous membrane of a small human bronchus shows a ciliated cell (CC) with cilia (C) and microvilli, a goblet cell (GC) with an apical mucous plug (MU), basal cell (BC), fibres and fibroblasts (FB) in connective tissue and macrophages (MP). The bronchi SEM micrograph of the epithelial surface shows ciliary tufts (C) and a mucous plug (MU) of a goblet cell in the process of extrusion. The distal lung TEM and SEM micrographs of the structure of the alveolar septum in human lungs show a septal fibroblast (FB), capillary endothelium (EN), alveolar epithelium (EP) and fibre strands (F). HE histology scale bars: 100 μm; bronchi TEM scale bar: 5 μm; bronchi SEM scale bar: 10 μm; distal lung TEM scale bar: 2 μm; distal lung SEM scale bar: 10 μm. Electron microscopy images reproduced and modified from [129, 130] with permission.

might therefore be an ideal solution as the biologically conducive nature of the ECM-derived scaffold material, such as sites for cell adhesion and organisational and differentiation cues, could be combined with synthetic materials and advanced manufacturing approaches to produce more reproducible products with tunable or controllable mechanical properties.

Cell types and scaling-up cell culture methods

In addition to identifying methods to reproducibly manufacturing suitable scaffolds for lung tissue engineering using either acellular or manufactured scaffolds, an additional challenge is in identifying cell sources and obtaining sufficient cell numbers. Ideally, autologous cells from the patient would be used for recellularising lung scaffolds in order to minimise post-transplantation immune complications and

the need for immunosuppressive medications. However, ideal GMP-grade manufacturing methods for mass production of the different cells likely needed for lung bioengineering are currently not widely established.

Fully differentiated primary adult cells and/or autologous endogenous lung progenitor cells could be a potential source. However, when using such an approach, multiple cell types would likely need to be isolated from the patient, grown to sufficient numbers *ex vivo* and introduced into the scaffold in the correct locations in order to generate functional lung tissue.

Although our understanding of what a "fully differentiated" or adult cell type is in the lung is constantly evolving, the use of terminally differentiated cell types would not allow for repair of the lung following injury. Therefore, endogenous progenitor cells, known to participate in adult repair processes, may be a more reasonable approach. One potential approach for regenerating the multiple cell types in the lung could be to seed multiple progenitor cell populations into the lung scaffold with the ultimate goal of directing differentiation into the different cell types found in the adult lung. This would avoid the necessity of having to introduce each different cell type into the lung and direct it to its correct anatomical location. Two exciting recent reports demonstrated that sufficient numbers of endogenous epithelial progenitor cells could be sourced from a single patient and expanded *ex vivo* to cover either tracheal grafts or a large extent of whole acellular human lung lobes [64, 107]. It is critical that these expansion methods are reliant on substances that are permissible for clinical use in humans [107]. Further development is needed to generate scalable methods using GMP-grade manufacturing approaches to ensure sufficient epithelial, endothelial or mesenchymal cell coverage. An additional limitation may also lie in identifying suitable endogenous progenitor cell populations from patients with existing lung disease. This may be challenging as there is emerging evidence that these cells are aberrant in chronic lung diseases [108–110]. However, this will be an interesting line of future research as previous reports indicate that the scaffold source influences cell behaviour more strongly than cell origin [26]. Thus, a normal scaffold may help revert the phenotype of cells obtained from diseased patients.

An alternative approach to endogenous progenitor cells is iPSCs. These are of particular interest as recent work has demonstrated that human iPSCs can be differentiated into cells expressing a distal pulmonary epithelial cell phenotype and seeded into acellular human lung scaffolds [59, 61]. Moreover, in patients with lung disease caused by known gene alterations, such as cystic fibrosis (CF), iPSCs derived from these patients could be gene-corrected prior to subsequent recellularisation [63, 111]. However, iPSCs also have several potential disadvantages: currently a patient skin biopsy is typically used to make iPSCs, but it has been shown that these cells partially retain the epigenetic memory of their tissue of origin [112]. This, while not yet explored in detail, could lead to limitations when differentiating iPSCs into specific lung lineages. Nonetheless, several studies have shown that iPSCs derived from fibroblasts can be used to derive cells containing phenotypic and functional markers of mature lung epithelial cell types [61–63, 113, 114]. Other studies have also examined the use of ESCs and found that these can be differentiated towards different adult epithelial phenotypes *ex vivo* [60, 66]. Despite their promise, ESCs carry ethical concerns, and both ESCs and iPSCs have been shown to form teratomas [115, 116]. Thus, both ESCs and iPSCs need to be further optimised and thoroughly investigated prior to clinical use.

Recent reports show promise for large-scale production and culture of cells using suspension culture (stirring) bioreactors and rotating wall bioreactors [61, 117], including techniques compliant with GMP and thus clinical use [35, 117–119]. This could allow for translation of stem cell research to clinical and pre-clinical applications modelling a potential GMP workflow. However, large-scale production of cells will need to be optimised for each cell type. Despite these advances, it is evident that new methods that decrease cost and time will be needed for cost-effective and less labour-intensive large-scale production of cells for clinical use.

Bioreactor strategies for lung bioengineering

Although the study of perfusion and ventilation is more straightforward when using acellular, intact lungs, there are still relatively few reports that have addressed these parameters [131–133], in large part due to the limited availability of suitable lung bioreactors. Various bioreactor strategies have been developed in recent years for lung and airway bioengineering approaches or reconditioning of lungs that were rejected for transplantation [7, 134]. Ghaedi *et al.* [135] developed a rotating bioreactor that exposed cells seeded onto thin slices of acellular lung to air and liquid alternately, which resulted in alveolar epithelial cell expansion. For de- and recellularisation of human-sized lungs, Gilpin *et al.* [50] developed a pressure-controlled apparatus where cell seeding was performed *via* gravitational force. A commercially available isolated lung perfusion system called the Organ Regenerative Control Acquisition bioreactor (Harvard Apparatus Regenerative Technologies, Holliston, MA, USA) has also been used by others for decellularisation [33, 34, 136]. Despite the advances made in the last few years with EVLP systems, the maximum amount of time that a healthy lung can be maintained *ex vivo* and transplanted is in the range

of 6 h [137–139]. It is thought that bioengineering lungs *ex vivo* will require a far longer period of maturation time in a bioreactor prior to transplantation. An emerging approach for bioengineering of the upper airways, where mostly horizontal or upright bioreactors have been utilised before [140], is to use the human body as a bioreactor. For instance, Delaere and co-workers [141, 142] bioengineered an allogeneic donor trachea first at the forearm of the recipient and then replaced the damaged trachea with the allograft. Attempts for *de novo* generation of organs also include the use of humanised animals such as pigs as bioreactors [143, 144]. Interestingly, a recent report using native porcine lungs in a cross-circulation model reported an *ex vivo* period of 36 h, but transplantation was not evaluated as an outcome [5]. Regardless of the *ex vivo* approach, ensuring that the developing lung or airway tissue receives sufficient nutrients and is developing properly will be important to examine in more detail in future studies (table 1). Incorporation of real-time measurements such as glucose, lactate, electrolytes, pH and mechanical ventilation properties in addition to vascular resistance will be important to understand the necessary *ex vivo* culture time and help to design smarter bioreactor strategies.

Regulatory and ethical implications for translating lung bioengineering approaches

Despite the recent and exciting advances made in lung bioengineering, a number of significant regulatory, ethical and practical challenges will need to be addressed for any of these technologies to enter the clinic on a larger scale. Each set of challenges (*e.g.* classification of regenerative medicine products, approval of materials, use of stem cells, *etc.*) will be unique to the bioengineering approach used. When developing these new potential therapeutic approaches, it is critical that these translational concerns are addressed as early in the product development pipeline as possible. Establishment of regulatory frameworks and GMP standards for tissue-engineered products and the enforcement of these is necessary to prioritise patient safety [145, 146]. Additionally, it remains completely unknown as to what evaluation criteria should be used for bioengineered lung tissue before the first-in-man clinical trials could be performed, but the use of parameters comparable to those used in EVLP may be a good first indication [147]. Moreover, the use of an appropriate large animal model that examines both short- and long-term outcomes will be necessary before these approaches are translated to the clinic. It will be critical for academic researchers, clinicians, industry and regulatory bodies to work with one another to establish these new frameworks.

To date, the majority of studies that have used human lungs for decellularisation have utilised lungs that do not meet the clinical criteria for transplantation or, alternatively, lungs from normal patients undergoing autopsy for non-lung-related death. Human tissue access for biomedical research differs significantly between different countries [148–150]. Therefore, if de- and recellularisation is to be realised on a larger scale and in the clinic, regulatory frameworks will need to be permissible for these technologies in each country. One potential unexplored source for generating acellular human lung scaffolds could be from DCD donors. DCD donors are used in many countries, but not all countries permit their usage due to ethical concerns. In most countries, DCD lungs are evaluated using EVLP prior to transplantation. However, a large portion of DCD donors are not used for transplant as they fail to reach the minimum criteria necessary for transplantation (primarily low blood gas values) after being evaluated *ex vivo* [151]. If all of these sources of human lung tissue can be used and bioengineering strategies can be designed to reproducibly generate functional lung tissue for transplantation, the biggest limiting factor will likely become the cost of manufacturing bioengineered lungs for all of the patients on waiting lists.

Although synthetic materials are not limited by these same ethical and regulatory restrictions with regard to identifying a suitable source, a major hurdle for the use of synthetic materials is the time and overall cost required for a material to receive European Medicines Agency or US Food and Drug Administration approval, which includes thorough characterisation of *in vitro* and *in vivo* material properties [152, 153]. Moreover, the regulation of stem cell and regenerative medicine products is a rapidly evolving area that varies greatly by country. In the last few years, countries such as Japan [154] have adopted new regulatory pathways that differ dramatically from the traditional paths to translation that have long-existed in most countries [150]. Therefore, the ability to conduct pre-clinical trials and ultimately the path to the clinic will be different in each country.

Discussion and outlook

Although significant progress has been made in bioengineering lung or airway tissue *ex vivo* with the ultimate goal of transplantation (table 2), most of the proof-of-concept studies for lung bioengineering have focused on endothelial and epithelial compartments. In order to generate functional lung tissue that will be able to function long term, the more than 40 different cell types and perhaps hundreds to thousands of different cellular subtypes will likely need to be recapitulated. However, it is currently not clear which of these cell types are essential to produce lung tissue that can minimally function *in vivo*. In one recent and innovative approach, the issue was raised as to whether all cells should be removed during

decellularisation. Dorrello *et al.* [155] developed techniques to selectively decellularise the epithelium, but retain the endothelium. Such a technique could be employed either *ex vivo* or *in vivo*. It is known that an intact vascular network is critical for transplantation as well as for maintaining the blood–gas barrier and allowing for proper graft function, but also for supporting the regenerative cells [156]. Removing only epithelial cells and retaining the vascular endothelium might be an option to increase the success of re-seeding and implantation for certain patients. However, in chronic lung disease patients where the vasculature is severely damaged (*e.g.* pulmonary arterial hypertension (PAH)), this may not be a suitable approach.

One major intriguing question in the use of acellular lung scaffolds is the challenge of recellularising the interstitial spaces and the importance of innervation of smooth muscle cells. Will the cells seeded into decellularised constructs extravasate through the existing basement membranes? Will reseeded fibroblasts or smooth muscle cells simply "find their way" to the appropriate anatomical location if seeded through the airways or the vasculature? How critical is innervation, and how will this be achieved within the scaffold and integrated into the recipient nervous system? Although manufacturing techniques such as 3D printing might help overcome some of the challenges with regard to spatially depositing cells, there are other challenges such as incorporating and controlling multiple nozzles with each cell type and generating structures at length scales capable of gas exchange.

It will be important to more fully understand the behaviour of seeded cells into acellular, synthetic or hybrid scaffolds and any subsequent remodelling of the ECM or immune response. It is currently unclear how "clean" a scaffold needs to be for lung tissue engineering approaches. Evidence from pre-clinical studies [157] and clinical use of other acellular scaffolds indicates that the processing of acellular scaffolds can influence their immunogenicity [46, 158]. In general, immune cells have not been introduced into any *ex vivo* bioengineering schemes; therefore, immunogenic responses would be limited to those that are a result of the recipient's immune system. Immune cells have been found to be able to induce pathological responses in fibroblasts seeded on acellular lung scaffolds, indicating their potential role in directing the fate of cells seeded on acellular scaffolds [159]. Interestingly, immune cells, such as macrophages, have recently been found to play a prominent role in directing normal alveolar regeneration *in vivo* [160]. Thus, the role of different immune cell populations in directing *ex vivo* regeneration and their potential role in regulating *in vivo* regeneration will be an interesting area of future investigation. Some tolerable amount of immunogenic proteins might help facilitate regeneration and maturation into functional tissue for transplantation during the *ex vivo* phase of maturation. Lung transplant recipients are currently placed on broad-spectrum immunosuppressive agents because a wide range of different immune cell populations (neutrophils, B-cells, T-cells, macrophages, *etc.*) have been implicated in lung transplant rejection, both acute and chronic rejection [30]. Whether or not this will be needed to the same extent for transplantation of a bioengineered lung is not yet known.

In addition, it is critical that future studies in the field more fully characterise and understand the potential functionality of bioengineered lung tissue (table 1). In clinical lung transplantation, patient demographics such as age, smoking history, absence of chest trauma and absence of malignancy are important criteria for selecting suitable donors, while functional criteria for donor lungs are ABO compatibility, clear chest radiograph, arterial oxygen tension (P_{aO_2}) >300 mmHg on inspiratory oxygen fraction (F_{IO_2}) 1.0% and positive end-expiratory pressure (PEEP) 5 cmH_2O [161]. For lungs reconditioned with EVLP, the current functional criteria differs between centres (*e.g.* Toronto, Lund or "OCS" (Organ Care System) protocol) [162]. Thus, one could envision that the minimum functional criteria for a bioengineered lung would be P_{aO_2} >350–400 mmHg on F_{IO_2} 1.0% and PEEP 5 cmH_2O, which is what is used for EVLP assessment [163]. Other standardised approaches for evaluation could be: blood gases from each bioengineered lobe, bronchoscopy, lactate dehydrogenase values, reactive oxygen species production, bronchoalveolar lavage analysis (of protein and inflammatory cytokines) and surfactant evaluations.

Although patent vasculature and gas exchange may be the most critical initial functions for evaluating whether a transplant could proceed, and have thus been the focus of previous studies [65], a bioengineered lung tissue and the cells within should ideally also be able to respond to the environment, meaning that other functions are likely necessary for longer-term functionality. Among these on the epithelial side are ciliary beating and clearance of inhaled particles and allergens, mucociliary clearance, and surfactant production. On the endothelial side, the ability of constructs to support events such as haemostasis and leukocyte extravasation in the context of lung injury will be important to explore in future studies. More broadly, any tissue-engineered construct should have the ability to respond appropriately to potential challenges and injuries, and to repair locally. Thus, how the recipient immune system may repopulate transplanted lung tissue remains almost entirely unexplored and may be incredibly important, especially given emerging evidence that the lung harbours haematopoietic progenitor cells and is a major site of platelet biogenesis in the body (up to 50% of total platelet production) [164]. Furthermore, it remains incompletely understood how recruited, monocyte-derived macrophages differ from tissue-resident

macrophages in the lung [165], but they have been shown to be important for regeneration of the distal lung [160]. Whether or not these and other immune cells will need to be present or intentionally seeded in an *ex vivo* regeneration scheme remains unknown.

Currently, there are five major types of patients who receive the majority of lung transplants, *i.e.* chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), PAH, CF and α_1-antitrypsin deficiency (AATD) patients. In all chronic lung diseases (*e.g.* COPD, IPF and PAH), there are known defects in the endogenous lung progenitor cell populations. Thus, in the absence of gene correction or modulation with exogenous factors, it is unclear if these cell types can be used in a lung bioengineering scheme. iPSCs might therefore be considered the most promising source of cells for a clinically relevant scheme. However, in both CF [63, 166] and PAH [167], mutations are also found in iPSC-derived lung epithelial progenitor and endothelial cells, respectively. Recent techniques have shown that iPSC-derived lung epithelial progenitor cells from CF patients can be gene-corrected *ex vivo* [63]. Due to the differences in potential cell sources for patients with specific lung diseases, it is clear that the bioengineering approach will not be a "one size fits all" solution and will need to be modified for each of the different major groups of lung transplant patients, and perhaps for each patient if individual mutations need to be gene-corrected.

In addition to approaches that aim to generate single or double lungs, there could be clinical impact in reconstructing single lobes or only pieces of lung tissue. Although single- or double-lung transplant is standard, transplantation of single lobes is only performed in some centres, but could be beneficial for certain patient groups, such as COPD and AATD patients [168]. Furthermore, single-lobe transplant from an adult donor has been performed in paediatric patients where size matching is challenging with adult donors [169]. Thus, bioengineered single lobes could be of use. Clinical conditions where only a portion of the parenchyma or airway is affected (*e.g.* trauma, local bronchiectasis, tracheal collapse and nonsmall cell lung cancer) could benefit from these approaches. Techniques to manufacture alveolar-like structures [82, 85, 86, 127], tracheal supports [99, 100] and replacements [146] have all been developed recently. Furthermore, there has been work on the development of biomaterials that can serve as artificial pleuras for diseases and acute conditions that affect the visceral pleura (*e.g.* pneumothorax and mesothelioma) [170, 171]. Thus, there may be potential clinical impact for partial reconstruction or replacement of lung tissue with tissue-engineered products in patients where only certain regions of the lung are damaged.

The pursuit of lung bioengineering approaches for regenerating lung tissue for transplantation has opened new opportunities for *ex vivo* modelling of different lung diseases. Although there has been less research on the use of synthetic materials in modelling lung disease, this is an emerging area of research with great potential to help model new aspects of lung disease that current models cannot address. Acellular lungs derived from patients with chronic lung disease more completely recapitulate the clinical heterogeneity and more faithfully mimic the clinical disease. In the pursuit of bioengineering lung tissue for transplantation, the approaches developed can also be used for performing drug screens, replacement of animal models, and better understanding of different lung diseases and regeneration. However, the majority of studies have been performed under simplified conditions that do not completely recapitulate the *in vivo* scenario. Future incorporation of immune cells as well as physiological parameters such as ventilation, perfusion and control of oxygen levels should be examined.

Tissue engineering of (complex) organs was once thought to be restricted to the realm of science fiction. However, exciting recent advances in the tissue engineering field are supportive of the idea that these technologies may indeed one day lead to therapies for patients with devastating and debilitating lung diseases. The recent significant advances made in lung tissue engineering have been largely due to the collaboration between biologists, chemists, material scientists, clinicians and engineers in both academia and industry; the realisation of lung tissue and airway engineering technologies in the clinic will be contingent on the continued evolution and successful integration of these fields.

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