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Lung bioengineering: advances and challenges in lung decellularization and recellularization

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Abstract

Purpose of review—Bioengineering the lung based on its natural extracellular matrix (ECM) offers novel opportunities to overcome the shortage of donors, to reduce chronic allograft rejections, and to improve the median survival rate of transplanted patients. During the last decade, lung tissue engineering has advanced rapidly to combine scaffolds, cells, and biologically active molecules into functional tissues to restore or improve the lung's main function, gas exchange. This review will inspect the current progress in lung bioengineering using decellularized and recellularized lung scaffolds and highlight future challenges in the field.

Recent findings—Lung decellularization and recellularization protocols have provided researchers with tools to progress toward functional lung tissue engineering. However, there is continuous evolution and refinement particularly for optimization of lung recellularization. These further the possibility of developing a transplantable bioartificial lung.

Summary—Bioengineering the lung using recellularized scaffolds could offer a curative option for patients with end-stage organ failure but its accomplishment remains unclear in the short-term. However, the state-of-the-art of techniques described in this review will increase our knowledge of the lung ECM and of chemical and mechanical cues which drive cell repopulation to improve the advances in lung regeneration and lung tissue engineering.

Keywords

decellularization; lung regeneration; lung scaffold; recellularization

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Conflicts of interest

There are no conflicts of interest.

INTRODUCTION

Lung bioengineering and tissue regeneration is an exciting and rapidly progressing area in the biomedical field. The increasing demand for transplantable organs in end-stage lung failures [1] has motivated researchers to investigate creative alternatives. One such approach involves the cellularization of natural extracellular matrix (ECM) lung scaffolds obtained via decellularization, a strategy that might allow researchers to leverage a large pool of lungs currently not suitable for transplantation [2,3]. The decellularization process consists of removing all cellular content from the scaffold while preserving its complex natural three-dimensional structure, the protein and molecular composition, and the macromechanical and nanomechanical properties of the source tissue. The decellularized scaffolds will then ideally support colonization of patient sourced stem/progenitor cells, and produce a transplantable, bioengineered lung, with significantly reduced risk of rejection and immunosuppression requirements [4,5].

Over the last several years, investigators have combined scaffolds, cells, and biologically active molecules into semifunctional tissues able to perform some level of short-term gas exchange, the primary function of the lung. However, full gas exchange and the creation of long-lasting bioartificial lungs remains an elusive goal. This review will discuss the current progress and challenges in lung decellularization and recellularization based on the available techniques that have and will continue to contribute to increased knowledge of lung ECM, chemical composition, and mechanical characterization.

REFINING THE TECHNIQUE: DECELLULARIZATION AND RECELLULARIZATION

There is a substantial literature on lung decellularization and recellularization [6,7■,8]. However, intermediate steps for ECM characterization, devices for tissue development, and functional/quality characterization are continuously evolving.

Decellularization

A variety of approaches have been utilized including one or more of a combination of physical [9], enzymatic, and chemical agents [10] (Fig. 1a). The most common decellularization protocols are detergent-based and include combined use of nonionic and ionic detergents, Triton X-100 and sodium deoxycholate (SDC) [7■,11] or anionic surfactant such as SDS, alone [6,12]. There is no standardized procedure, in part as the lungs, in contrast to other organs, can be perfused with decellularization solutions through either the airways, the vasculature, or both simultaneously; all three approaches have been utilized. Moreover, different concentrations of each reagent, specific order of administration, and routes of perfusion are described in the literature [8,13,14]. Further refinements [15] have attempted to define the most effective way to perfuse these lungs. For example, some approaches have utilized either physiological flow or pressure through the vasculature [6,16], whereas others have utilized incremental perfusion rates, systematically alternated between airway and vasculature [11,17]. Moreover, different lengths of exposure times for

detergent and other decellularization reagents, ranging from several hours to several days, have been utilized [10,13].

Characterization of the decellularized scaffold

The decellularization technique is particularly challenging requiring a balance between preservation of the structural and chemical composition of the ECM (Fig. 1b) with total cellular content removal (Fig. 1c). Despite the different protocols, most investigators achieve some degree of decellularization, which can be defined as [7■,18] macrostructure and microstructure preservation, minimal residual DNA, preservation of the native ECM composition, and preservation of relevant mechanical properties. Suggested criteria for successful decellularized include absence of cellular content and nuclear material by routine histologic (Hematoxylin & Eosin, DAPI) staining levels of double-stranded DNA below 50 ng/mg dry tissue, and absence of DNA fragments above 200 bp on DNA gels [19]. Based on our experience, using Triton X-100 and SDC preserves the structure while removing cellular components better than methods using SDS or CHAPS detergent (3-((3-cholamidopropyl) dimethylammonio)-1-propane-sulfonate). Pressure-controlled decellularization protocols pose the risk of insufficient delivery of detergents to atelectatic areas of the lung especially in larger organs from pigs and humans. Small lungs from rodents for example are therefore usually easier to decellularize. As the diffusion length during the incubation steps is shorter even improper detergent distribution can usually be overcome. We believe that volume controlled methods are a better approach standardizing the amount of liquid used to the tidal volume of the respective organ to be decellularized.

Visualization of the decellularized scaffold and assessment of structural integrity generally involves light microscopy, immunofluorescent staining of residual ECM proteins such as collagens, elastin, laminin, fibrillin, or either ECM components including glycosaminoglycans (GAGs), and electron micrographic techniques [20]. Although histologic and immunofluorescent approaches provide important information on overall decellularized lung structure, electron microscopy, while providing important ultrastructural datais limited to a few individual regions and does not give an overall evaluation of the scaffold in its entirety [9,21].

The chemical composition of the ECM is commonly assessed by immunofluorescent staining and quantitative measurements of specific structural components. However, the use of quantitative techniques such as western blotting is limited to the assessment of few selected proteins [6,7■,11,22]. Moreover, the ECM composition can vary depending on the protocol that has been utilized [7■,23,24■■]. Therefore, more quantitative methods like protein arrays [25] and mass spectrometry with semiquantitative and quantitative proteomics [23,24■■,26■,27,28■] are employed. These global approaches allow better comparative assessments of the residual protein content left behind [29■] by different methods of decellularization.

The mechanical properties of the decellularized lung scaffolds, including stiffness, elastance, airways resistance, and others, are critically important but not as well studied. Approaches utilizing mechanical ventilation and tensile strength testing of lung strips are the most commonly used approaches [12,30]. Recent investigations utilizing atomic force microscopy

have provided more detailed assessments of the nanomechanical properties of decellularized lung tissues [31,32] (Fig. 1d). However, further research in this field is needed.

Current decellularization approaches have proven the possibility to isolate the lung natural scaffold. To move lung tissue engineering toward clinical application based on acellular scaffolds all described methods for scaffold characterization (Fig. 1) need to be performed to certify the accomplishment of the procedure.

Recellularization

Before starting the recellularization process, it is necessary to assure that residual detergents are properly removed, as they are detrimental to cell survival. Available techniques to confirm proper detergent removal by invasive [6] and noninvasive detergent detection methods [33] should be widely used as quality control.

A wide variety of cell types have been seeded into decellularized models including rodent, pig, and human lungs. These have included mixtures of fetal lung homogenates, differentiated lung epithelial, pulmonary vascular endothelial, and stromal cells, and a variety of endogenous lung progenitor and stem cell populations including lung epithelial and other cells derived from induced pluripotent stem cells [2,3,11,34▪▪–36▪▪,37▪]. These have also included xenogeneic approaches, for examples, human cells into decellularized rat or pig lungs [38▪,39▪▪]. A variety of seeding strategies have been employed as well including pressure vs. flow drive airway vs. vascular inoculations. These approaches have resulted in a range of results showing ability to differentially seed different compartments (airways vs. vasculature) and have provided important information on bioreactor techniques necessary to sustain and promote cell growth [4,5,34▪▪,35▪▪,37▪,40▪,41,42▪–44▪]. However, there is no clear advantage to any particular type of seeding methods or range and combinations of inoculated cells. Further, the ultimate goal of recreating the lung cellular environment with properly functioning airway and alveolar epithelial cells as well as pulmonary vascular endothelial cells and stromal cells all in their proper compartments remains difficult to achieve. As such, decellularized lungs can certainly be populated with cells but creation of functional gas exchange units capable of long-term use remains as elusive today as it did following the first reports of these approaches in 2010 [3–5,36▪▪,42▪,45,46].

Further knowledge about the nature of the ECM is critical for improving recellularization as ECM affects cellular behavior including stem cell differentiation [25,47–49]. Moreover, the bioreactor technologies utilized to culture the recellularizing lungs, consisting of sophisticated isolation chambers, continuous perfusion systems, environmental control, and physiological ventilation, are continuously evolving [40▪,50].

There are numerous emerging applications stemming from this singular pursuit, already beginning to provide a return on these investments. These include the bioengineering of individual lung structures for transplantation, development of gas-exchange assist technologies, high-throughput three-dimensional tissue models, for testing promising drug and therapeutic strategies, research tool for studying lung developmental biology and disease mechanisms, material sourcing for development of lung ECM derived biomaterials [51,52].

APPLICATIONS, CHALLENGES, AND OPPORTUNITIES

The bioengineering of the whole-organ, particularly the lung is an exciting evolving field in biomedical engineering [37■,44■] (Fig. 2). The research related to lung tissue regeneration has rapidly progressed in recent years. However, the lung is a very complex organ composed of numerous cell types, chemical ECM components and intrinsic mechanical properties [53], and this complexity offers many challenges. For example, the most common techniques used to decellularize lungs involves perfusion of chemical and biological agents, often in combination with a physical method, which may alter or destroy important ECM components impacting the structure and the possibility for future cells to adhere and proliferate [54–56]. Currently understudied areas are GAG and lipid composition of scaffolds. GAGs are particularly important to investigate as they can bind matrikines and can therefore critically influence cellular behavior [28■]. New advanced bioreactor systems must be designed to provide the bioengineered lung with nutrition, oxygen, and mechanical ventilation, but also real-time measurements of for example electrolytes, pH, glucose, and lactate [50]. Standardized sterilization processes which do not negatively affect the cells or scaffolds need to be optimized [22,57–59]. Although a functional gas-exchange unit is the final goal, decellularized lungs can provide valuable research tools with which to study ECM and cell-matrix interactions in normal and in diseased lungs.

CONCLUSION

Eight years after the initial reports of bioengineered lungs, the decellularization techniques and ECM characterization continue to be refined as do modifications in cell seeding conditions and bioreactor technologies. Further investigations need to focus on large-area coverage, cell coculture, differentiation, surfactant production, and long-term gas-exchange to finally achieve transplantable bioengineered lungs based on decellularized and recellularized scaffolds.

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KEY POINTS

- The ECM, known as scaffold, can be obtained by the decellularization process maintaining three-dimensional structure, chemical composition, and mechanical properties.
- The acellular lung scaffold can be repopulated with different cell types particularly stem and lung progenitor cells. However, new approaches are needed in terms of complete surface coverage and gas-exchange.
- The interest in lung bioengineering is increasing in the regenerative medicine field, due to the current shortage of donors for organ transplantation and the possibility to develop engraftments obtained from allogenic and xenogeneic donors that can be translated into the clinics.
- Bioengineering the lung is at an early stage; however, initial results of gas-exchange are motivation to the scientific community to finally achieve transplantable bioengineered lungs.

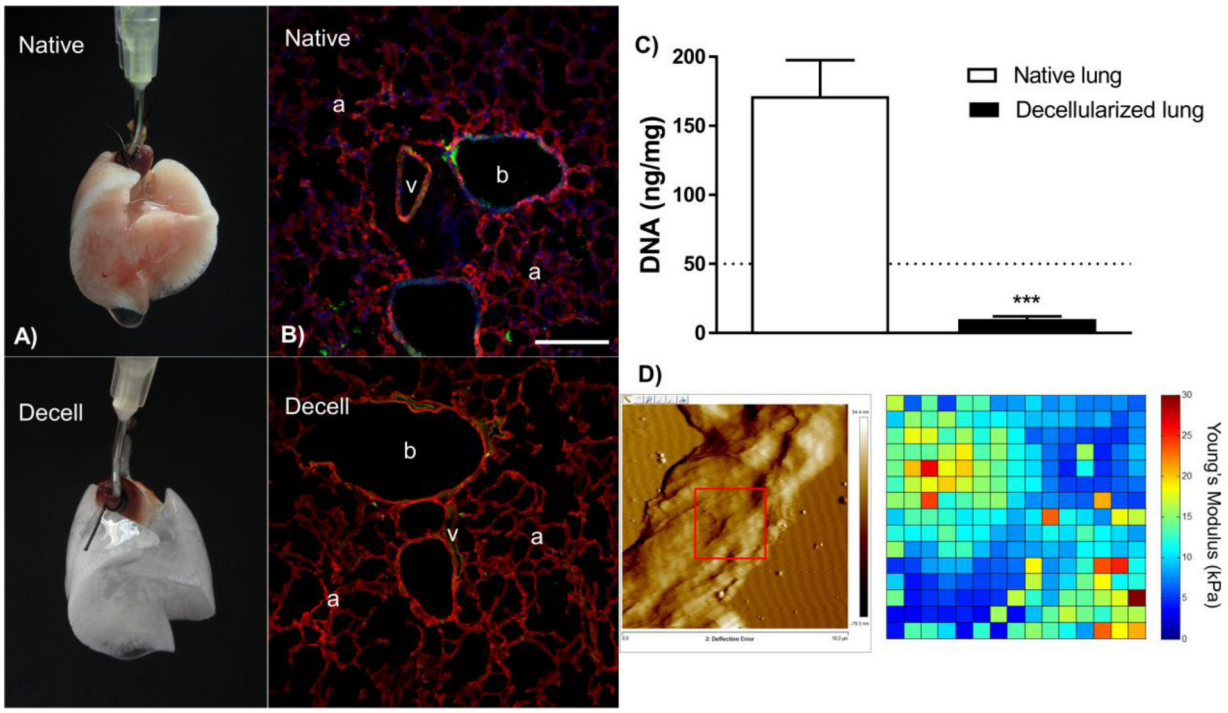

FIGURE 1. Characterization of the decellularized lung scaffold.

The panel depicts in (a) a native and a decellularized murine lung which preserves its three-dimensional structure. (b) Chemical composition of the extracellular matrix, assessed by immunofluorescence and confocal microscopy, determines that cell nuclear components are absent in the decellularized scaffold, whereas collagen I and elastin are present after the procedure. The bronchial space (b), vasculature (v) and alveoli (a) are depicted in the images. Bar scale is 200 μm. (c) The DNA content remaining after decellularization is below the threshold of 50 ng/mg of dry tissue total protein suggested in the literature [18]. Data are presented as means ± SD and n = 4. (d) The nanomechanical properties of the acellular lung scaffold are depicted by a topographical image and a force map obtained on an alveolar wall of the lung parenchyma. Pyramidal cantilever, nominal spring constant of 0.03 N/m. The panel is constituted by original images obtained in collaborative work of D.J.W. at the University of Vermont and Prof Dr R. Farré at the University of Barcelona.

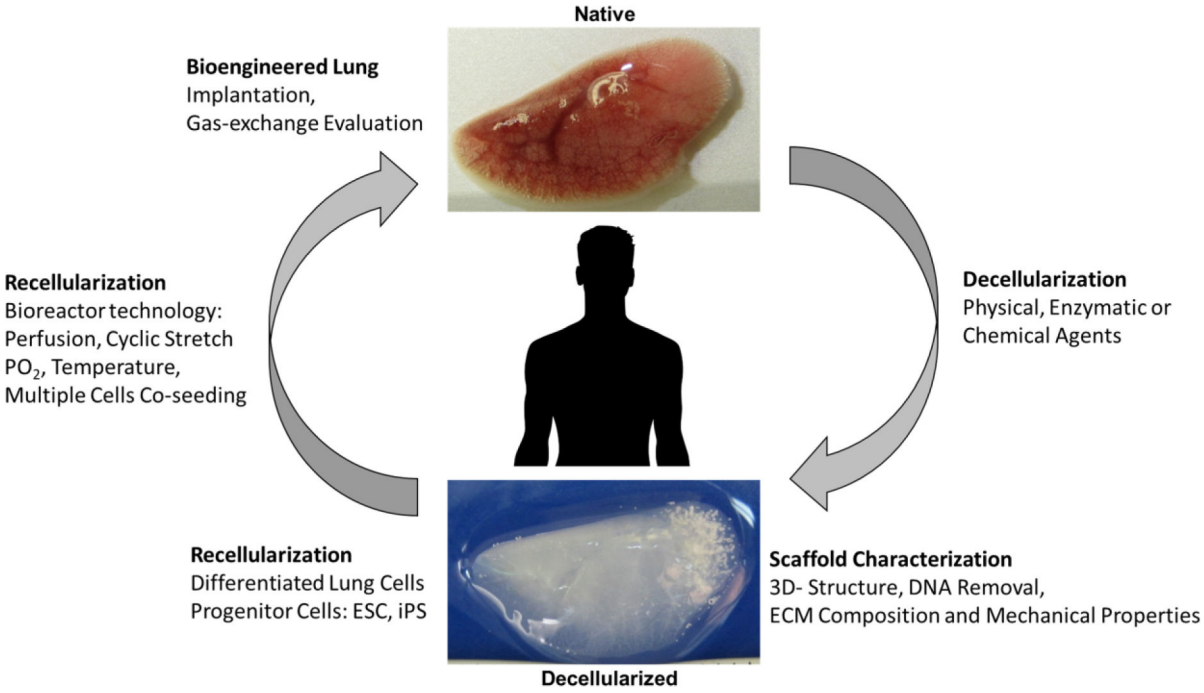

FIGURE 2. Procedure to bioengineer the lung.

Schematic for the procedure for bioengineering the lung based on the decellularization and recellularization process. This figure is based on previous published work [4].