Bioengineering Lungs for Transplantation

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KEYWORDS

• Tissue engineering • Lung regeneration • Epithelium • Endothelium • Organ culture
• Bioartificial lung

KEY POINTS

- Whole lung extracellular matrix scaffolds can be created by perfusion of cadaveric organs with decellularizing detergents, providing a platform for organ regeneration.
- Lung epithelial engineering must address both the proximal airway cells that function to metabolize toxins and aid mucociliary clearance and the distal pneumocytes that facilitate gas exchange.
- Engineered pulmonary vasculature must support in vivo blood perfusion with low resistance and intact barrier function and be antithrombotic.
- Repopulating the native lung matrix with sufficient cell numbers in appropriate anatomic locations is required to enable organ function.
- The combination of mechanical, chemical, and biological stimuli can be applied to the regenerating organ during ex vivo culture in a bioreactor, allowing for testing of the maturing lung prior implantation.

INTRODUCTION

Lung transplantation remains the only curative treatment option for most advanced lung diseases.[1] Unfortunately, a substantial shortage in the availability of healthy lungs from cadaveric donors for transplantation exists. This reality is aggravated by the prevalence of cigarette smoking and associated chronic obstructive pulmonary disease, which results in an increasing demand for new therapies and donor organs.[2] Additional clinical indications for lung transplantation include idiopathic pulmonary fibrosis, cystic fibrosis, and primary pulmonary hypertension.[3] Compounding the problem, a relatively low lung utilization rate from organ donors, reported at less than 30%, contributes to long wait times for transplantation.[4] Most frequently, lung nonutilization is a result of poor organ function at the time of donation. One promising approach to improve donor lung function and transplantability is the ex vivo lung perfusion system (EVLP [XVIVO Perfusion AB, Göteborg Sweden]).[5] This technology to recondition donor lungs and restore acceptable function criteria has resulted in an expanded donor pool and an increase in total lung transplant recipients.[6] Nevertheless, this approach does not directly address the long-term issues associated with lung transplantation, which most significantly include chronic rejection and the development of bronchiolitis obliterans syndrome.[7] As a means to overcome this immune response to allografts, tissue and organ engineering proposes to create transplantable organs by combining biologically suitable scaffolds with a patient's own cells. These implantable constructs are presented as a theoretic alternative to cadaveric donor organs for transplantation, which may ultimately eliminate the need for lifelong immunosuppression and provide long-lasting therapeutic benefits to patients in need. The challenges of scaffold production, optimal cell choice for regeneration, and the appropriate design of biomimetic culture systems are discussed.

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THE SCAFFOLD: DECELLULARIZED NATIVE EXTRACELLULAR MATRIX

In order to engineer solid organs with the biological complexity of their native counterparts and the capacity to regain appropriate physiologic functions following transplantation, the foundation on which to build is an important consideration. Much evidence has demonstrated that the process of whole organ decellularization can provide an ideal framework for regeneration. By accessing the organ's native vascular network, detergents or other recellularizing agents can be delivered in a controlled and extensive manner throughout the organ, first lysing the endogenous cellular elements and then effectively removing the resulting debris. Whole, perfusion-based lung decellularization was first reported in rodents,[8,9] validating the concept and providing an initial proof-of-concept framework for generating whole organ scaffolds. Commonly used detergents include the anionic sodium dodecyl sulfate and sodium deoxycholatedeoxycholate and the zwitterionic detergent, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate. The minimal criteria proposed to confirm adequate decellularization are less than 50 ng dsDNA retained per milligram extracellular matrix (ECM) dry weight, less than 200 bp DNA fragment length, and visible loss of nuclear material following DAPI (4′,6-diamidino-2-phenylindole) or H&E (hematoxylin and eosin) stain.[10] Deeper analysis of the resulting lung matrices generated following detergent perfusion has demonstrated preservation of the native organ architecture, supported by the retention of essential extracellular proteins.[11,12] Detailed proteomic analysis by mass spectrometry confirmed that decellularized lung matrix is predominantly composed of structural collagens, laminin, and elastin and also retains varying degrees of proteoglycans and glycoproteins.[11,13,14] Maintenance of intact structural compartmentalization has also been demonstrated for the acellular lung scaffolds, with a preserved basement membrane extending throughout the vascular network and delicate distal airspaces. The retention of these key biological elements, in the correct anatomic locations, is important to facilitate the eventual reintroduction of specialized cells types to the appropriate location. Initial biocompatibility of the scaffold in coculture with epithelial cells, endothelial cells, fibroblasts, and mesenchymal stem cells has been demonstrated.[9,15]

To translate the decellularization procedure to a clinically relevant size, the initial methodologies have been successfully up-scaled to porcine, macaque, and human donor lungs.[16,17] The ease of scaling this procedure to human-size organs provides an advantage over other manufactured scaffolds, including those made of crosslinked polyglycolic acid, collagen, or Gelfoam (Pfizer, New York, USA) which are not ideal for large-scale organ engineering.[18–20] Validation of the decellularized large lungs scaffolds has confirmed a similar preservation of organ architecture and matrix composition, further supporting the utility of this procedure for clinical-scale bioengineering.[11] The structure of the native lung is largely determined by the complex network of matrix and connective tissue in functional mechanical units.[21] Maintaining this microarchitecture within the decellularized scaffold is required for subsequent reacquisition of essential organ biomechanics. Following cell removal and any accompanying cell-derived materials, such as glycosaminoglycans and pulmonary surfactant, the dynamic mechanics of the system are dramatically altered. Precise measurement of whole organ biomechanics using traditional pulmonary functions tests, such as compliance and resistance, is challenging when applied to an acellular scaffold.[22] Changes to the mechanical properties of decellularized lung tissue may vary depending on the detergent and decellularization approach used.[23] It is important to note that changes in local stiffness may not directly translate to alterations in global biomechanics, but may have significant effects on the cells repopulating a specific microenvironment.[24] The true consequences of lung decellularization on functional mechanics may be most appropriately assessed following recellularization and organ culture, as a critical test before implantation.

Thinking toward clinical application, the establishment of minimally acceptable criteria for a decellularized lung scaffold may be required, including ECM composition, bioburden, and immunogenicity. There are little currently reported data addressing the immune response of a recipient to decellularized lung matrix, which highlights a crucial area of required study. Another important consideration that requires further investigation is the source of donor organs. Scaffolds derived from individuals with known lung diseases, including emphysema and fibrosis, may retain biological cues that can negatively influence the behavior of reintroduced cells and recapitulate disease phenotypes.[25,26] Many questions remain regarding the effect of donor age and species when developing the optimal platform for regeneration. At minimum, the biological lung scaffold must be able to support the attachment and growth of various reintroduced cell types, without significantly altering their phenotype or inhibiting