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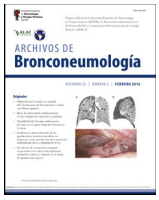

Review article

# Bioengineered Lungs: A Challenge and An Opportunity

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ABSTRACT

Lung biofabrication is a new tissue engineering and regenerative development aimed at providing organs for potential use in transplantation. Lung biofabrication is based on seeding cells into an acellular organ scaffold and on culturing them in an especial purpose bioreactor. The acellular lung scaffold is obtained by decellularizing a non-transplantable donor lung by means of conventional procedures based on application of physical, enzymatic and detergent agents. To avoid immune recipient's rejection of the transplanted bioengineered lung, autologous bone marrow/adipose tissue-derived mesenchymal stem cells, lung progenitor cells or induced pluripotent stem cells are used for biofabricating the bioengineered lung. The bioreactor applies circulatory perfusion and mechanical ventilation with physiological parameters to the lung during biofabrication. These physical stimuli to the organ are translated into the stem cell local microenvironment – e.g. shear stress and cyclic stretch – so that cells sense the physiological conditions in normally functioning mature lungs. After seminal proof of concept in a rodent model was published in 2010, the hypothesis that lungs can be biofabricated is accepted and intense research efforts are being devoted to the topic. The current experimental evidence obtained so far in animal tests and in *ex vivo* human bioengineered lungs suggests that the date of first clinical tests, although not immediate, is coming. Lung bioengineering is a disrupting concept that poses a challenge for improving our basic science knowledge and is also an opportunity for facilitating lung transplantation in future clinical translation.



## Pulmones biofabricados: un desafío y una oportunidad

*Palabras clave:*
Ingeniería tisular
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RESUMEN

La biofabricación de pulmones es un nuevo desarrollo en ingeniería de tejidos y medicina regenerativa destinada a proporcionar órganos que se podrían usar para trasplante. Se basa en la siembra de células en un andamio acelular del órgano y su cultivo en un biorreactor específico. El andamio acelular se obtiene descelularizando un pulmón no trasplantable por medio de procedimientos convencionales basados en agentes físicos, enzimáticos y detergentes. Para evitar el rechazo inmunitario del receptor del pulmón una vez trasplantado, en la biofabricación se usan células madre autólogas mesenquimales derivadas de médula ósea/tejido adiposo, células progenitoras de pulmón o células madre pluripotentes inducidas. El biorreactor aplica perfusión circulatoria y ventilación mecánica con parámetros fisiológicos al pulmón durante el proceso. Estos estímulos físicos al órgano se traducen en el microambiente local de la célula madre —por ejemplo, tensión de cizallamiento y estiramiento cíclico— para que las células perciban las condiciones fisiológicas típicas en el pulmón maduro con funcionamiento normal. Tras la publicación en 2010 de la prueba de concepto en un modelo de roedor, se ha aceptado la hipótesis de que los pulmones pueden ser biofabricados y se están dedicando grandes esfuerzos de investigación a este tema. Las pruebas experimentales obtenidas hasta ahora en ensayos con animales y *ex vivo* en pulmones humanos

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biofabricados indican que la fecha de las primeras pruebas clínicas, aunque no inmediata, se va acercando. La bioingeniería pulmonar es un concepto disruptivo que nos desafía a mejorar nuestros conocimientos científicos básicos y da una oportunidad para facilitar el trasplante de pulmón en una futura traslación clínica.



## Introduction

It goes without saying that there is a considerable need of lungs for transplantation, as indicated by the progressively increasing waiting lists of patients for lung transplant worldwide. One of the causes limiting the availability of suitable lungs is that a high number of donor lungs are not acceptable according to current quality control criteria. To solve this problem, research efforts are currently devoted to improve organ preservation procedures and donor management to rescue lungs that otherwise would be not suitable for transplantation.[1,2] These investigations are expected to result in increasing the number of lungs for transplantation.

This review is focused on a novel alternative procedure for obtaining organs for transplantation: lung biofabrication. This approach, which is still in its first experimental steps, has raised considerable expectancy and research efforts in recent years. Lung biofabrication is based on building a new lung by seeding an acellular organ scaffold with progenitor cells from the patient that would be the recipient of the regenerated organ. Such an "autologous" lung would avoid the difficulty of conventional allogeneic transplanted lungs to deal with immune rejection from the recipient, which is the main cause why lung transplantation has a success rate (~50% survival after 5–6 years) lower than in other organ transplant.[3] The following text is aimed at presenting the principles and methods of lung biofabrication, the current state of the art of the topic and its potential for future clinical translation.

## The Bioengineered Lung Approach

A new possible way to increase the availability of organs for transplantation is currently facilitated by recent progress in stem cell biology and in tissue engineering and regenerative medicine. Indeed, the hypothesis that tissues and organs can be biofabricated is currently accepted and, after proofs of concept have been published in the last years, intense research worldwide is focused on the topic. Although the terms "bioengineered" or "biofabrication" suggest that organs are built artificially, it should be mentioned that the current approach is mainly based on using natural components such as cells and extracellular matrix (ECM) scaffolds. In fact, the rationale is to replicate the natural process of organ development. Namely, an organ is built by precisely placing newly divided cells into a 3D structure where cells differentiate in an environment providing the chemical (e.g. nutrients, growth factors, cytokines, chemokines) and physical (e.g. temperature, oxygen pressure, stiffness) cues required by the cells.

Hence, organ/tissue biofabrication is based on the suitable combination of three core elements: cells, scaffolds and bioreactors, as illustrated by Fig. 1. Briefly, a donor lung which is not acceptable for transplantation is decellularized to eliminate the entire cell components from the lung. The resulting acellular lung scaffold, which maintains the 3D structural complexity of the native organ, is recellularized with new cells. The process of new organ biofabrication is carried out placing the recellularized scaffold into a bioreactor aimed at providing physico-chemical stimuli for optimal cell homing, proliferation and differentiation (Fig. 1).

### *Cells Employed in Lung Biofabrication*

The optimal cell types for organ biofabrication are still an open question. An obvious option could be to employ differentiated cells corresponding to the organ, for instance bronchial and alveolar epithelial cells for lung biofabrication. Although the simplest approach from a biological perspective, this option would not be feasible in routine applications since, as these differentiated cells virtually do not proliferate, a great number of donor organs would be required to biofabricate one single *de novo* organ. Taking into account the high proliferative and differentiation capacities of stem/progenitor cells, using these cells for repopulating acellular scaffolds is the preferred current approach.

The ideal option is to use autologous stem/progenitor cells to avoid immunoreaction of the bioengineered organ by the receptor. Adult stem cells, such as bone marrow- or adipose tissue-derived mesenchymal stem cells (MSCs) have the advantage of being easily obtained but suffer from the drawback that MSCs are not totipotent and thus cannot differentiate into all the cell phenotypes in a given organ (up to 60 in the lung).[4] The most promising option is to bioengineer organs with induced pluripotent stem cells (iPSCs). These cells, obtained by reprogramming adult cells into an embryonic-like stage, have full differentiation capacity and, if the donor and recipient are the same patient, iPSCs are not immunogenic. However, these cells are relatively new, with their long term fate unknown, and hence some open questions about potential clinical use are still open. In the specific case of lung biofabrication, almost all types of stem cells have been employed to advance our understanding of the mechanisms involved. In rodent lung biofabrication, seminal studies employed both adult differentiated epithelial/endothelial cells and embryonic stem cells. In more recent experimental works using human lung scaffolds, both bone marrow- and adipose tissue-derived MSCs, and human iPSC-derived pulmonary progenitor cells have been used.

### *Scaffolds for Lung Bioengineering*

In case of bioengineering tissues that are relatively simple from a structural viewpoint (e.g. bone repair, skin, heart valves, vessels) the scaffold can consist on artificial 3D substrates made by natural (e.g. collagen, chitosan) or artificial polymers (e.g. polyglycolic acid (PGA), polylactic acid (PLA)) using different techniques (e.g. solvent casting, fiber bonding, electrospinning).[5,6] Other current techniques to build 3D constructs composed of ECM and cells (e.g. using cell sheets[7] or bioprinters)[8] can be used for bioengineering structurally simple tissues/organs. Nevertheless, none of the currently available techniques, including the most advanced 3D printers, can provide the resolution required to mimic the structural details of a complex organ such as heart, lung, liver or kidney. For instance, the adult lung is composed of ~300 millions of alveoli, each with a diameter of ~300 μm. This airway tree is paralleled with a similarly complex circulatory tree since each alveolus is accompanied by blood capillaries with a typical diameter of ~8 μm. The alveolar-capillary membrane separating air and blood is extremely thin (~3 μm), presenting an effective gas exchange surface of 70 $m^2$. And all this structure is packed in a total volume