Journal Club Medecine-Science

Session III: Tissue Engineering

David PUYRAIMOND-ZEMMOUR, TCEM1 Oncology Paris VI
Eimad SHOTAR, TCEM1 Radiology Paris V
CRI, 10/27/2010

Introduction

- Organ transplantation is often the only treatment for final stage disease
- Shotcommings
  - Availability
  - The need for immunosuppression
- Tissue engineering

- Scaffold based airway and lung tissue engineering

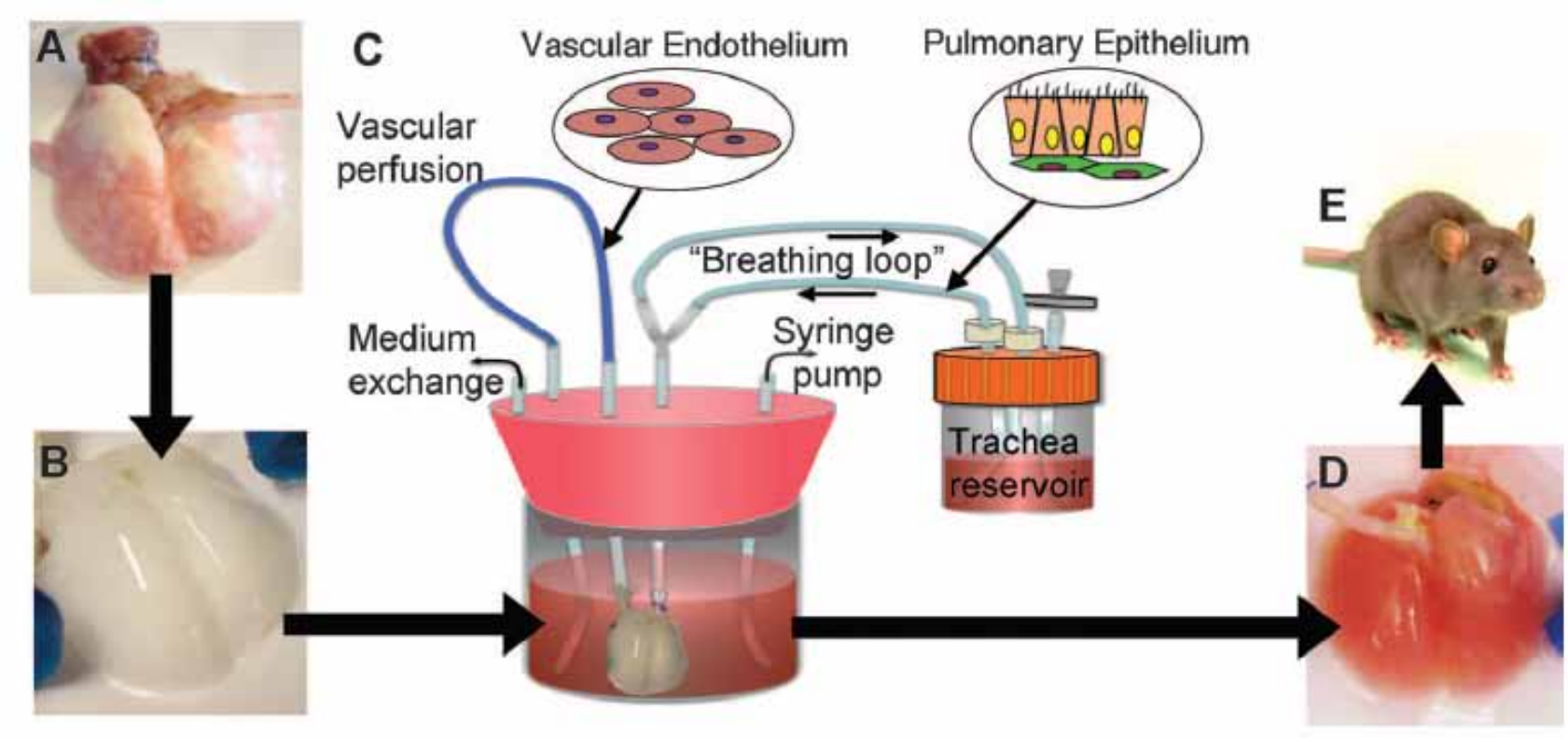
Tissue-Engineered Lungs for in Vivo Implantation

Thomas H. Petersen,[1,2] Elizabeth A. Calle,[1] Liping Zhao,[3] Eun Jung Lee,[3] Liqiong Gui,[3] MichaSam B. Raredon,[1] Kseniya Gavrilov,[4] Tai Yi,[5] Zhen W. Zhuang,[6] Christopher Breuer,[5] Erica Herzog,[6] Laura E. Niklason[1,3]*

30 July 2010 vol 329 Science

Tissue-Engineered Lungs for in Vivo Implantation

I Overall Strategy



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Tissue-Engineered Lungs for in Vivo Implantation

II Preparation of a Decellularized Lung Scaffold

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Tissue-Engineered Lungs for in Vivo Implantation

II Preparation of a Decellularized Lung Scaffold

- Lung Tissue was harvested from adult Fischer 344 rats
- The lungs were treated in the vascular and airway compartments with a **detergent solution**
  - 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)
  - In a phosphate buffer at 1.0 salt concentration
- Vascular perfusion was maintained < 20mmHg with exposure time 2-3h.

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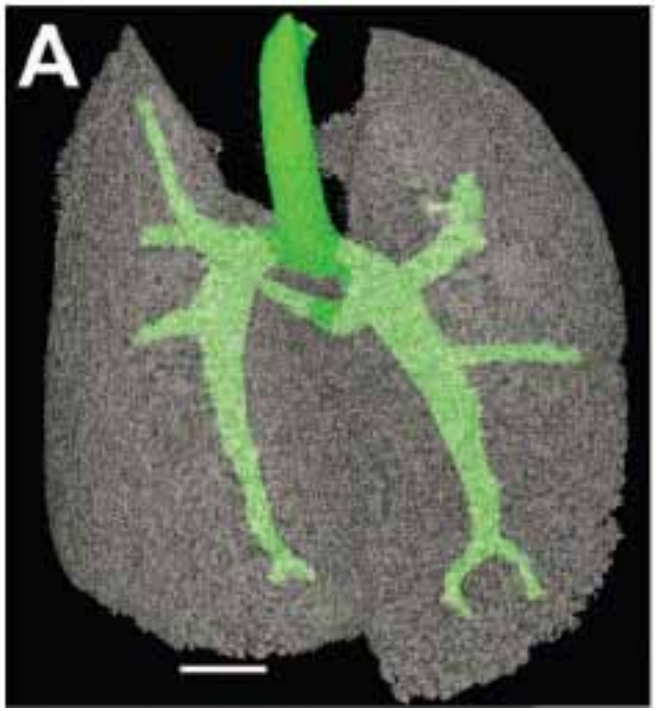

3D-microCT

- Intact Lung Architecture

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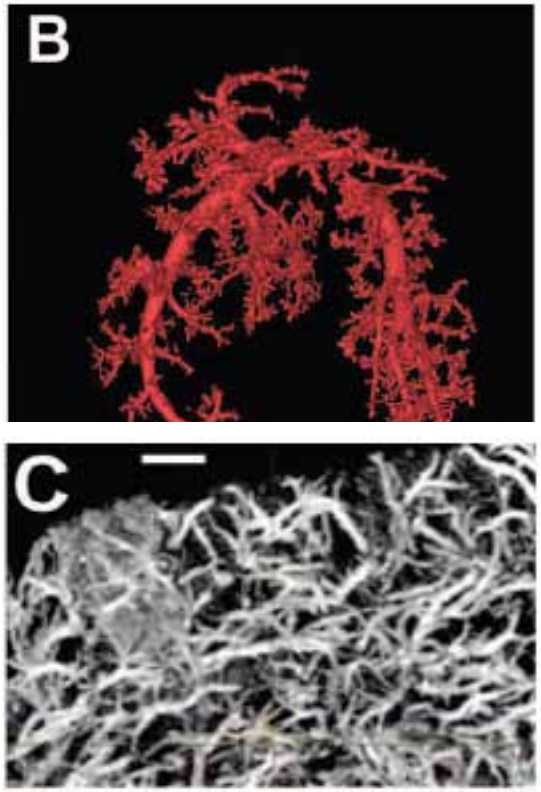

3D-microCT angiography

- Intact Lung Architecture
- Intact Arterial tree and microvasculature

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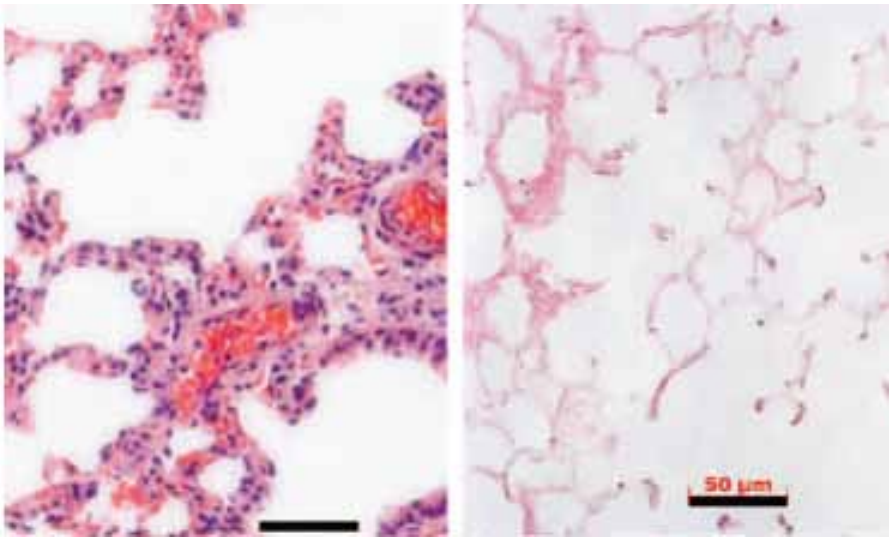
II Preparation of a Decellularized Lung Scaffold



Hematoxylin & Eosin staining

- Intact Lung Architecture
- Intact Arterial tree & microvasculature
- Cells & nuclear material removed
- Alveolas septal architecture conserved

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Tissue-Engineered Lungs for in Vivo Implantation

II Preparation of a Decellularized Lung Scaffold

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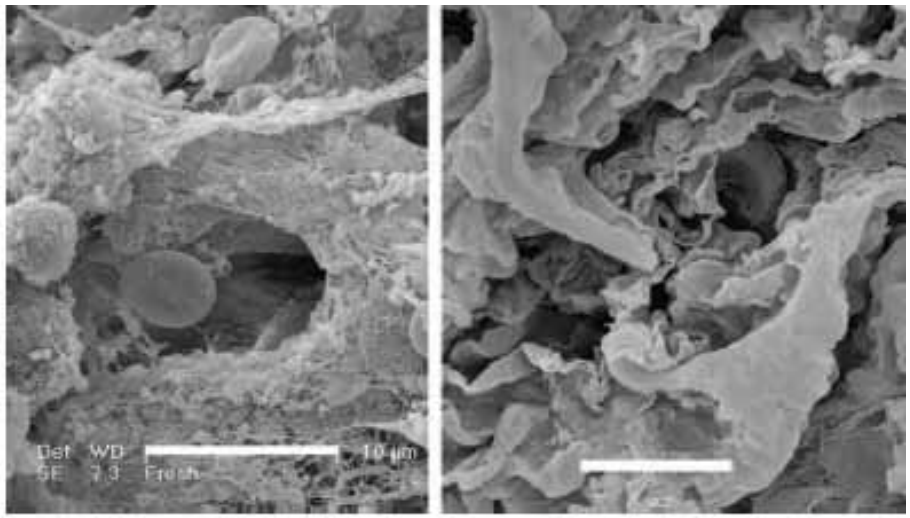

Scanning Electron Microscopy

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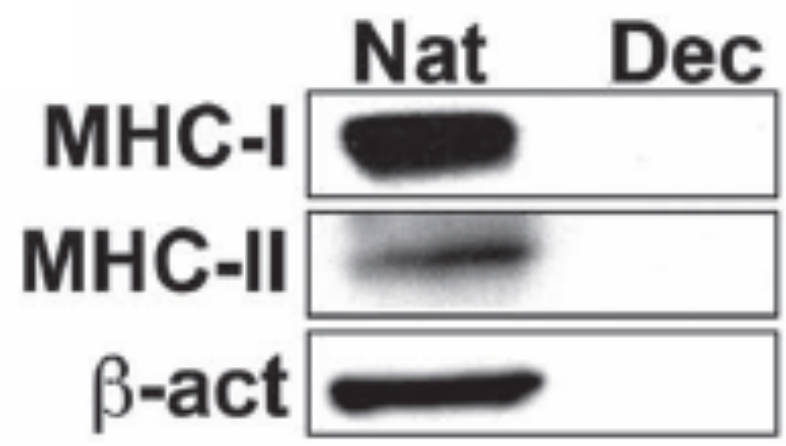
II Preparation of a Decellularized Lung Scaffold



Immunoblotting

- Intact Lung Architecture
- Intact Arterial tree & microvasculature
- Cells & nuclear material removed
- Alveolas septal architecture conserved
- Matrix was depleted of MHC I & II as well as βactin

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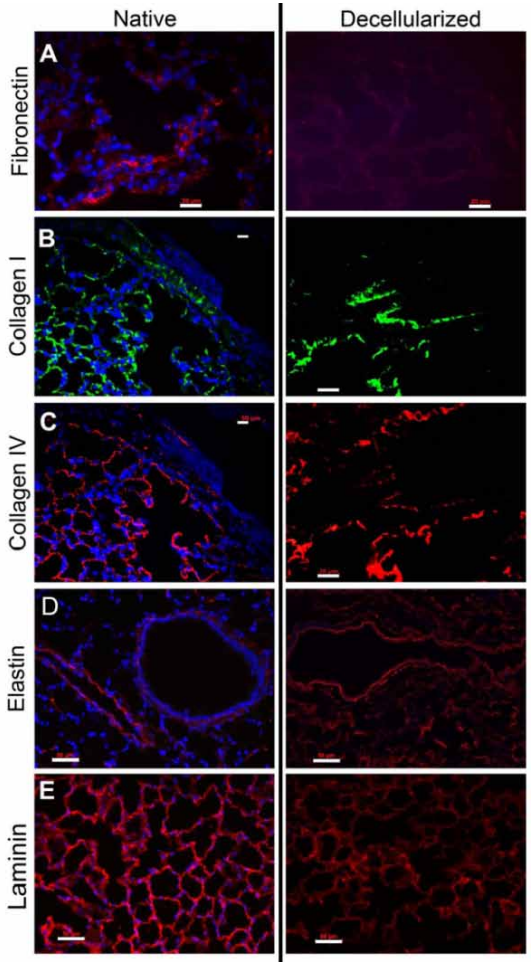
Tissue-Engineered Lungs for in Vivo Implantation

II Preparation of a Decellularized Lung Scaffold



- Intact Lung Architecture
- Intact Arterial tree & microvasculature
- Cells & nuclear material removed
- Alveolas septal architecture conserved
- Matrix was depleted of MHC I & II as well as βactin
- Matrix retains Collagen, Elastin and Laminin

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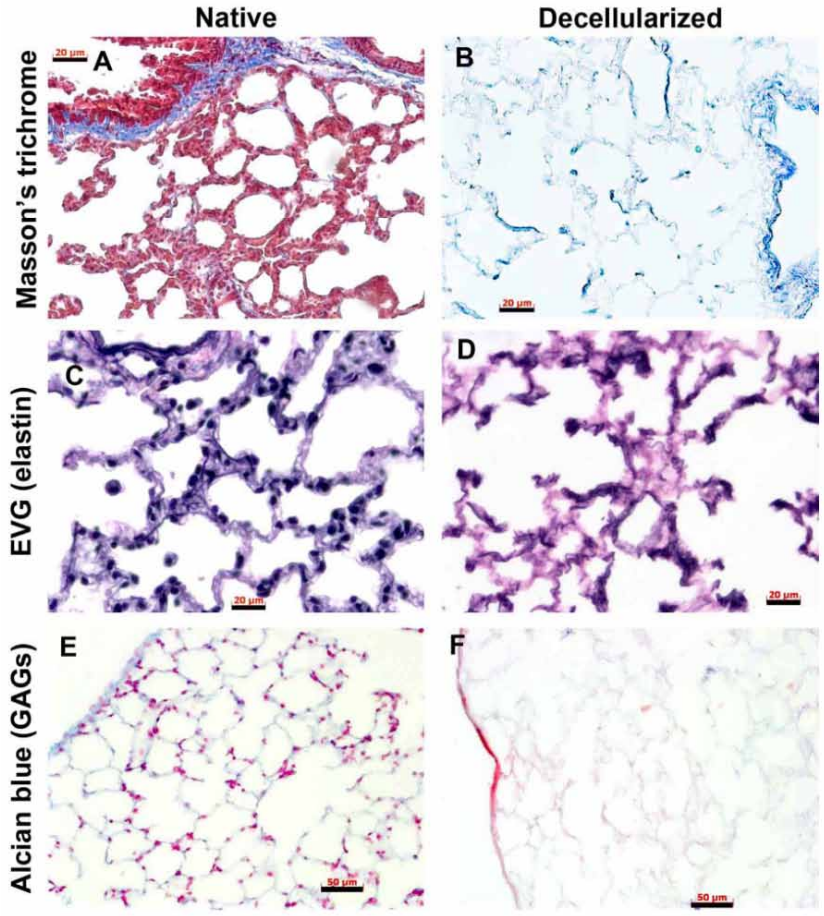
Tissue-Engineered Lungs for in Vivo Implantation

II Preparation of a Decellularized Lung Scaffold



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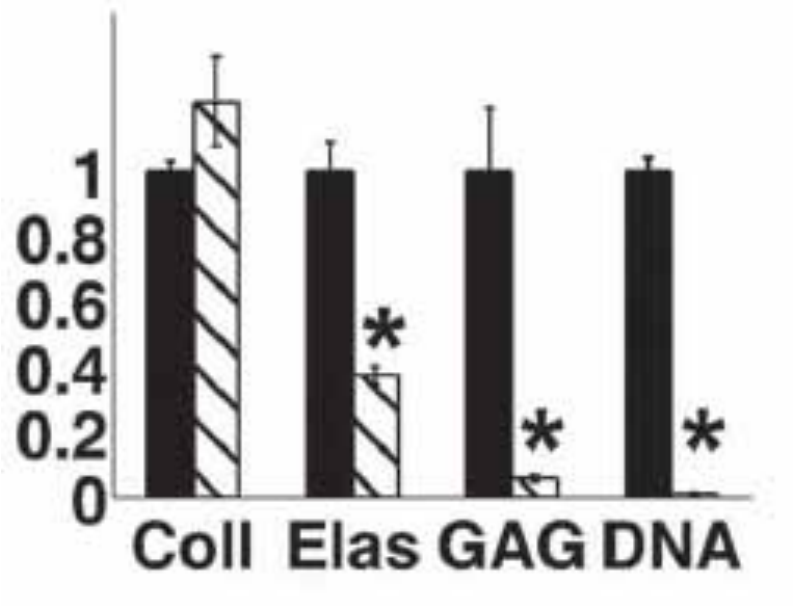

- Intact Lung Architecture
- Intact Arterial tree & microvasculature
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Tissue-Engineered Lungs for in Vivo Implantation

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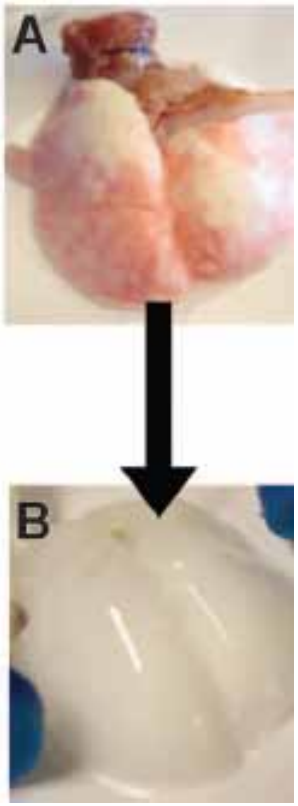

Hence, the lung **decellularization** protocol produces an acellular matrix **scaffold** that retains the gross, microstructural, and ultrastructural properties of native lung, yet **removal of antigenic cellular components** is essentially complete

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Tissue-Engineered Lungs for in Vivo Implantation

III Properties of the Lung Bioreactor

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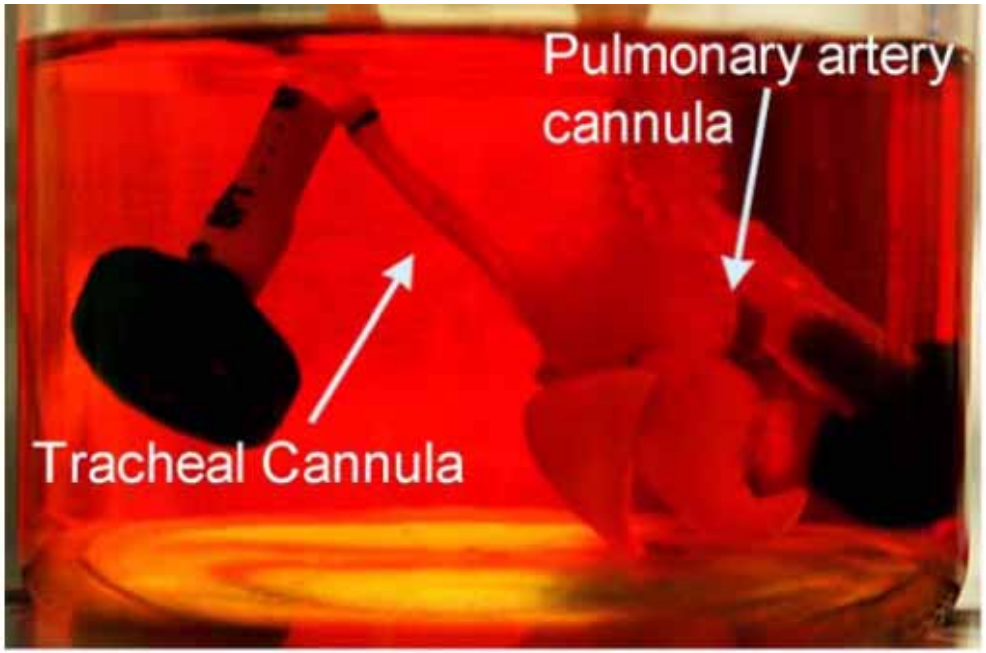
Tissue-Engineered Lungs for in Vivo Implantation

III Properties of the Lung Bioreactor



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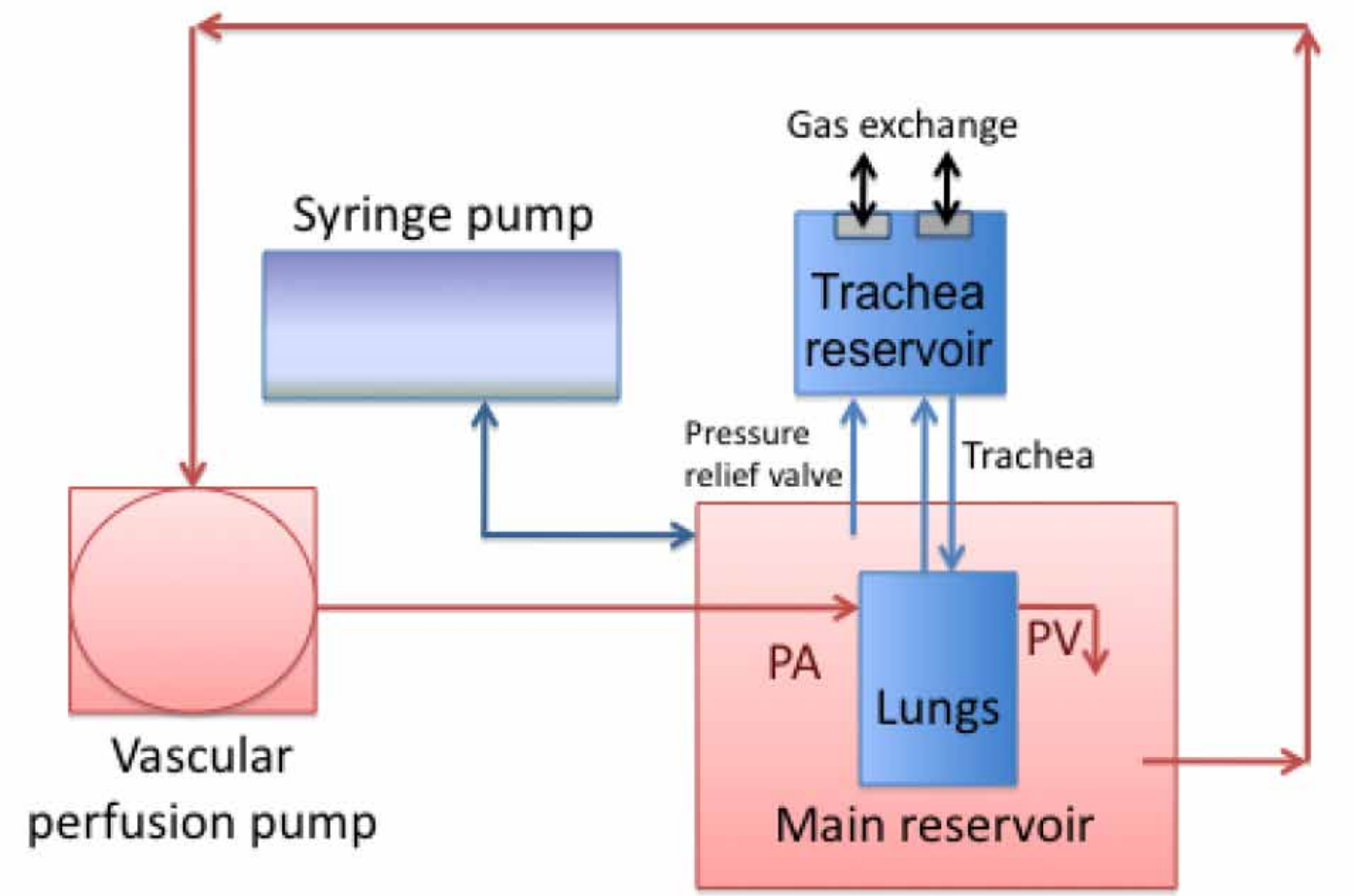

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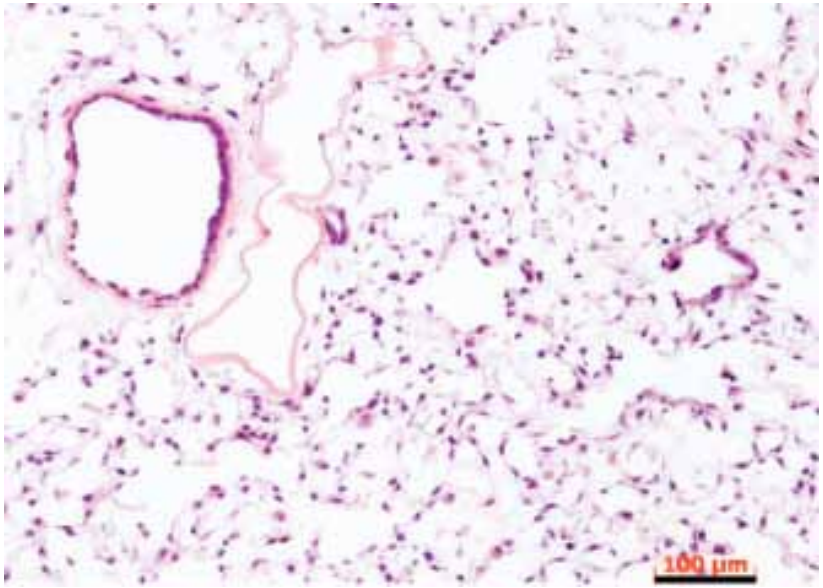
Tissue-Engineered Lungs for in Vivo Implantation

IV Repopulating the acellular matrix

- Lung epithelial cells were isolated from neonatal (<7 day-old) Fischer 344 rats
- The cells were seeded into the airway compartment



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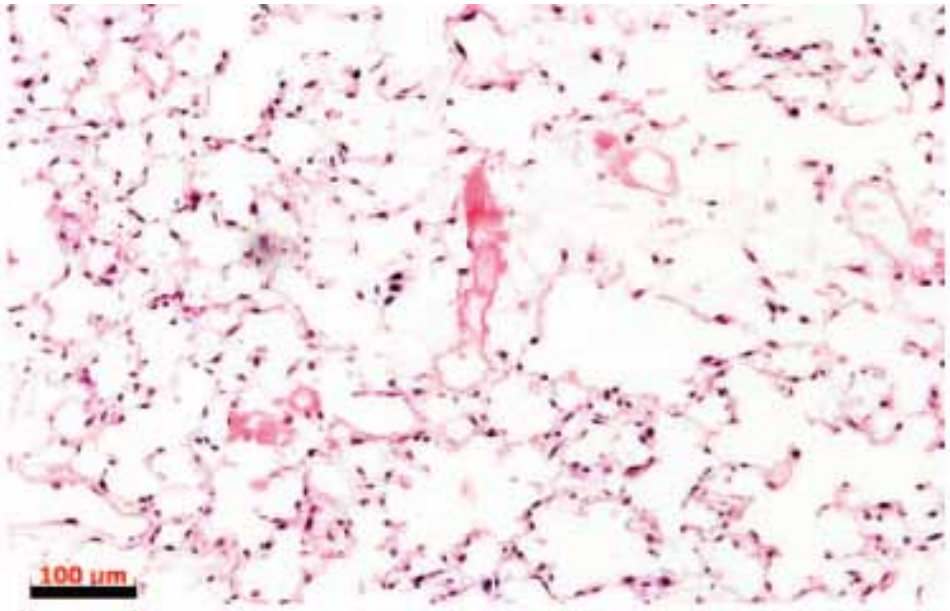
Tissue-Engineered Lungs for in Vivo Implantation

IV Repopulating the acellular matrix

- Rat lung microvascular endothelial cells (RMECs) were seeded into the vascular compartment



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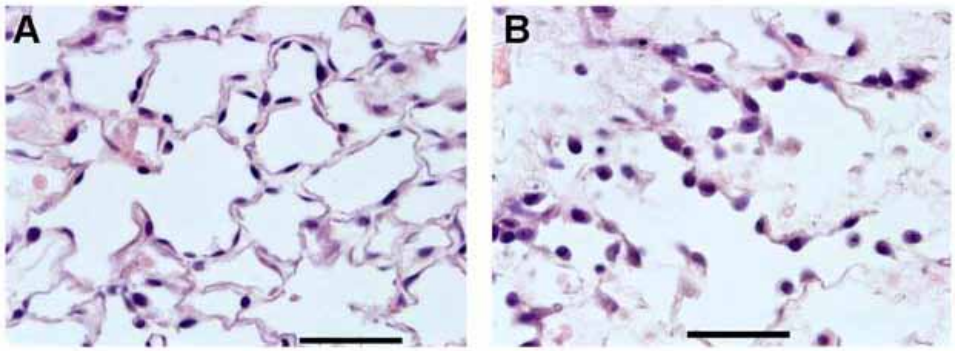
Tissue-Engineered Lungs for in Vivo Implantation

IV Repopulating the acellular matrix

- Bioreactor conditions enhanced repopulation



- Vascular perfusion enhanced endothelial adhesion

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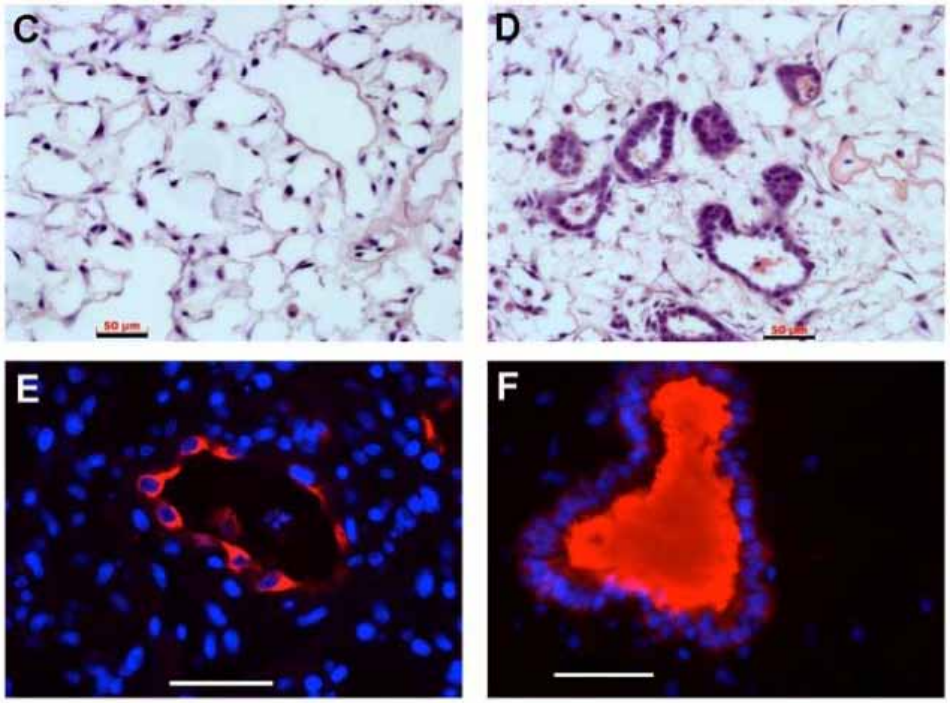
IV Repopulating the acellular matrix

- Bioreactor conditions enhanced repopulation



- Vascular perfusion enhanced endothelial adhesion
- Negative pressure ventilation
  - Enhanced adhesion
  - Enhanced clearance

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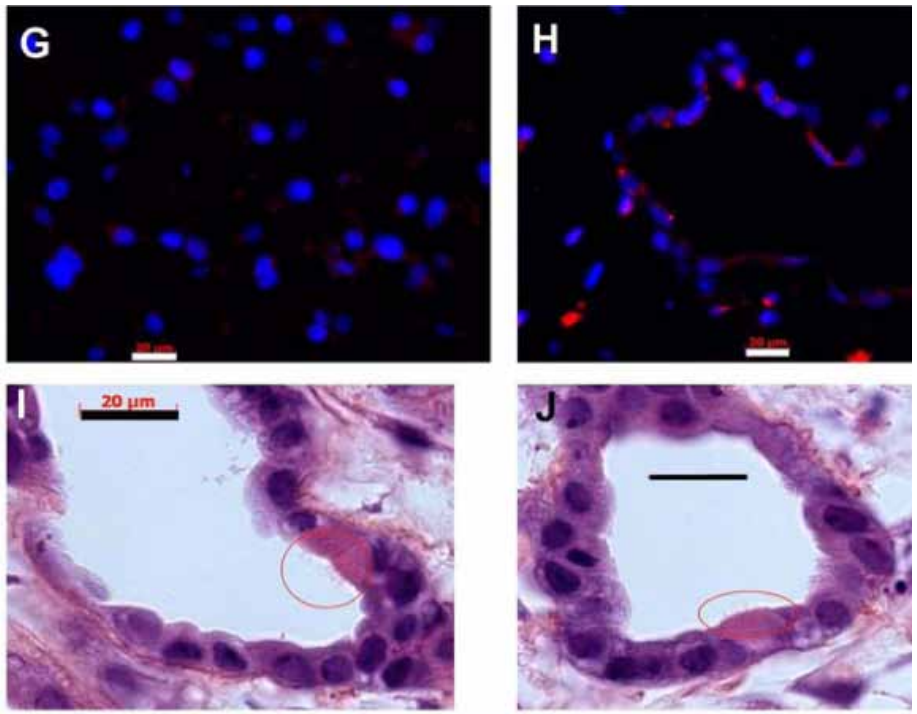
Tissue-Engineered Lungs for in Vivo Implantation

IV Repopulating the acellular matrix

- Bioreactor conditions enhanced repopulation



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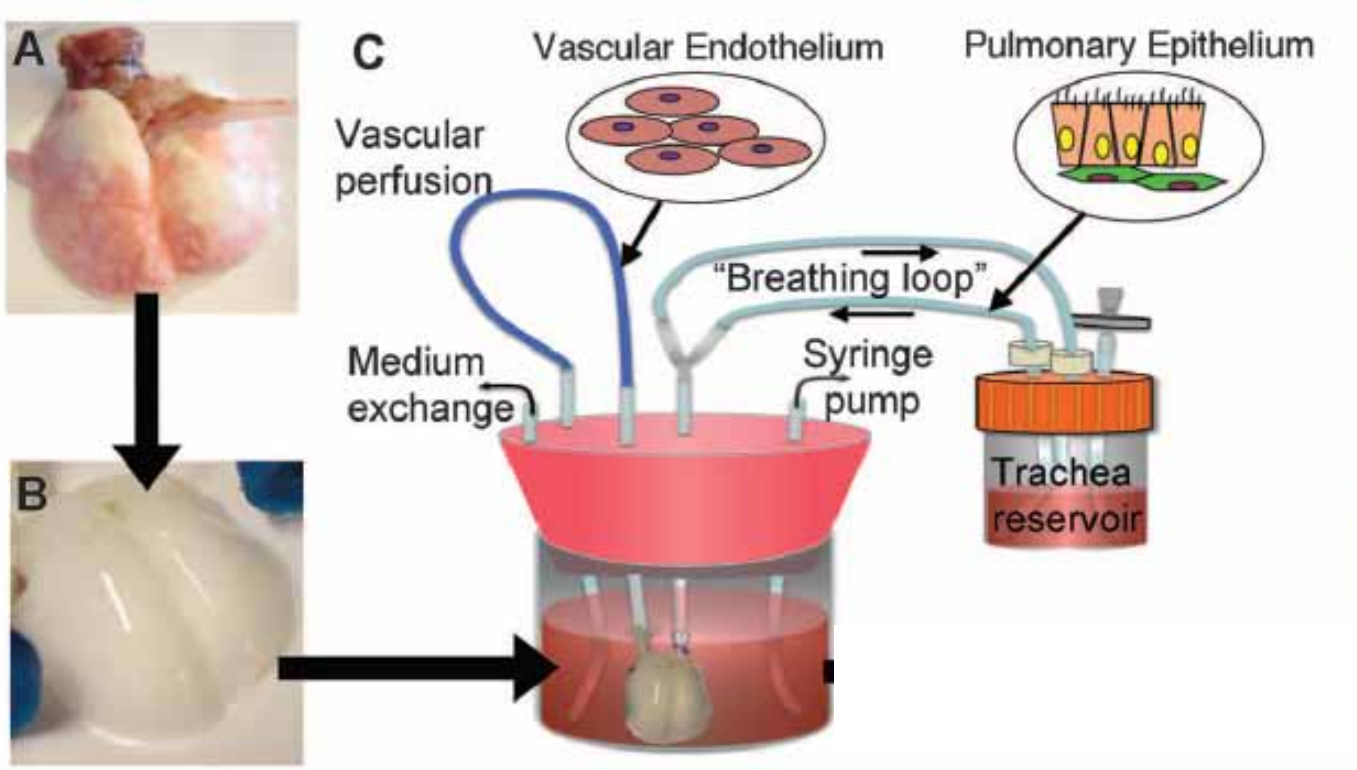
- Vascular perfusion enhanced endothelial adhesion
- Negative pressure ventilation
- Ventilation with air
  - Type I alveolar epithelial cells
  - Ciliated columnar c.

Tissue-Engineered Lungs for in Vivo Implantation

IV Repopulating the acellular matrix



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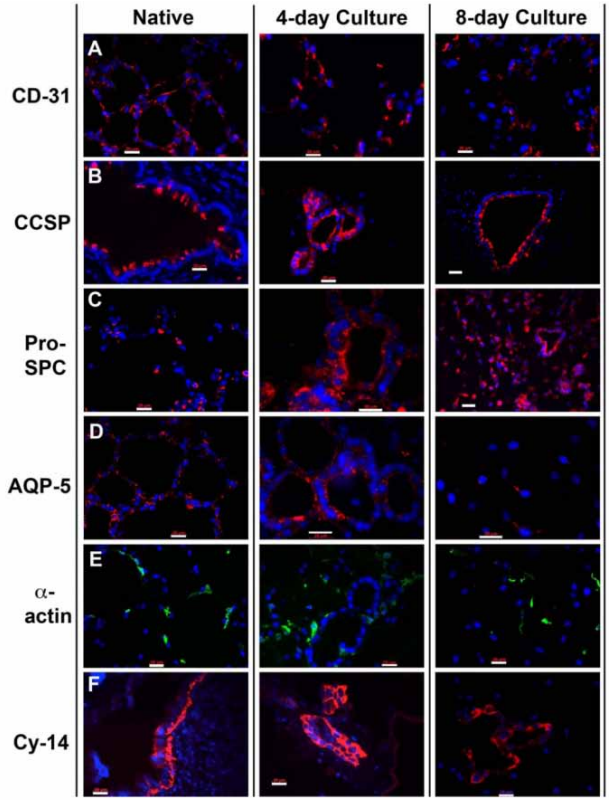
Tissue-Engineered Lungs for in Vivo Implantation

IV Repopulating the acellular matrix

- Characteristics of the Engineered lungs



- Cellular composition

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IV Repopulating the acellular matrix

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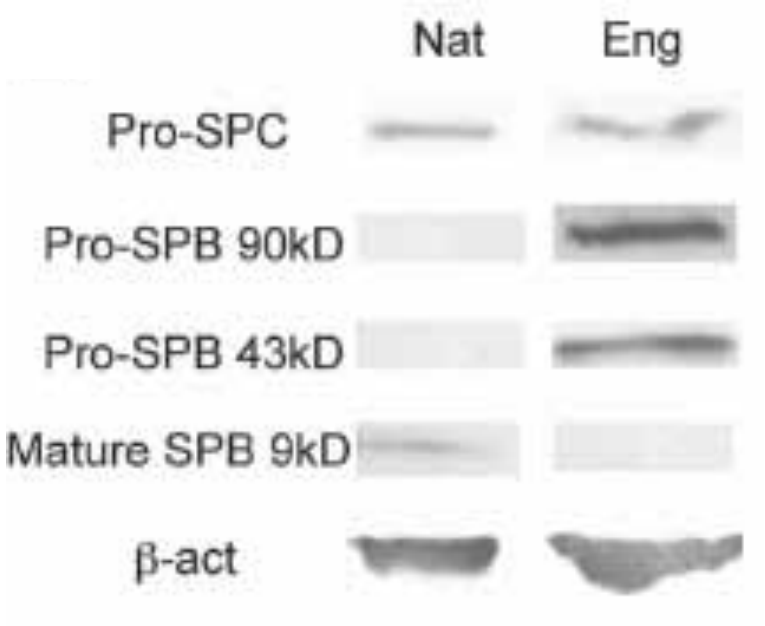

- Cellular composition
- Surfactant secretion

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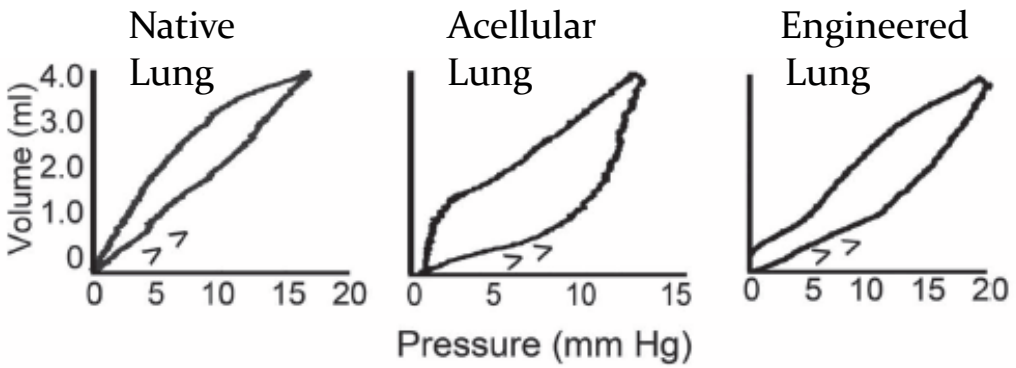
IV Repopulating the acellular matrix

- Characteristics of the Engineered lungs



- Cellular composition
- Surfactant secretion
- Mechanical properties
  - Compliance

$$C = \frac{\Delta V}{\Delta P}$$

  - Native 0.35 +_ 0.08 ml/mmHg
  - Acellular 0.09 +_ 0.02 ml/mmHg
  - Engineered 0.14 +_ 0.06 ml/mmHg

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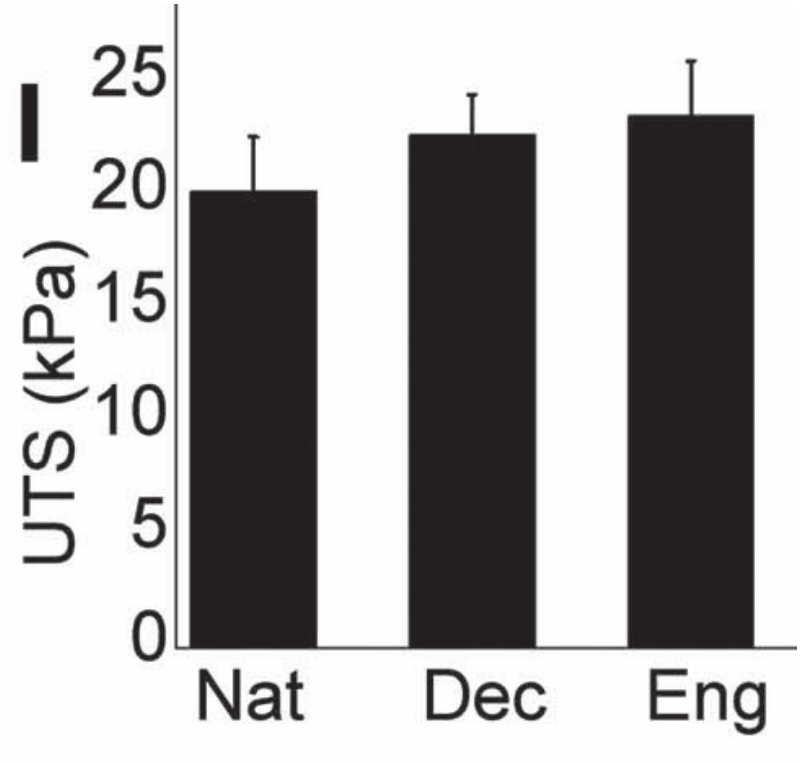
IV Repopulating the acellular matrix

- Characteristics of the Engineered lungs



- Cellular composition
- Surfactant secretion
- Mechanical properties
  - Compliance
  - Ultimate tensile stress

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Tissue-Engineered Lungs for in Vivo Implantation

V Implantation of Engineered Lungs into Rats

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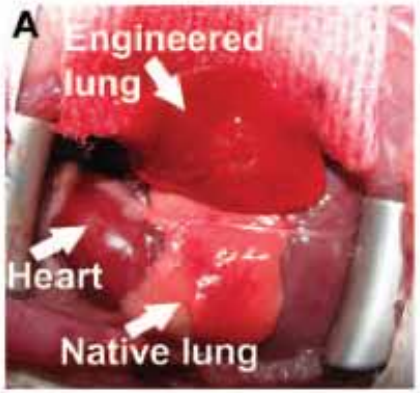
Tissue-Engineered Lungs for in Vivo Implantation

V Implantation of Engineered Lungs into Rats



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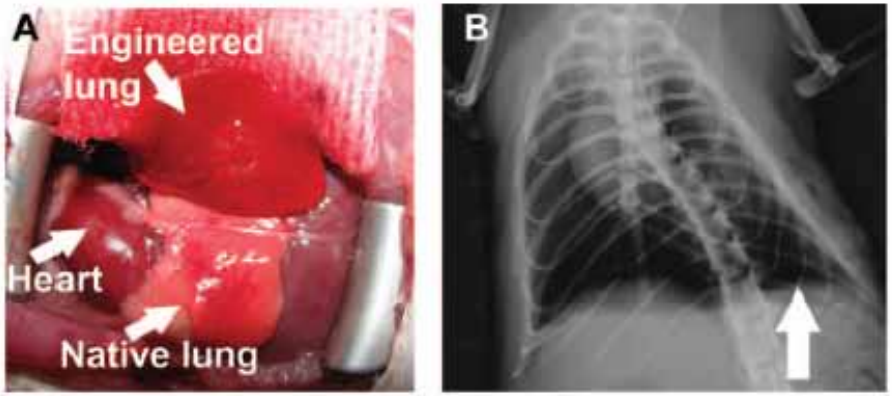

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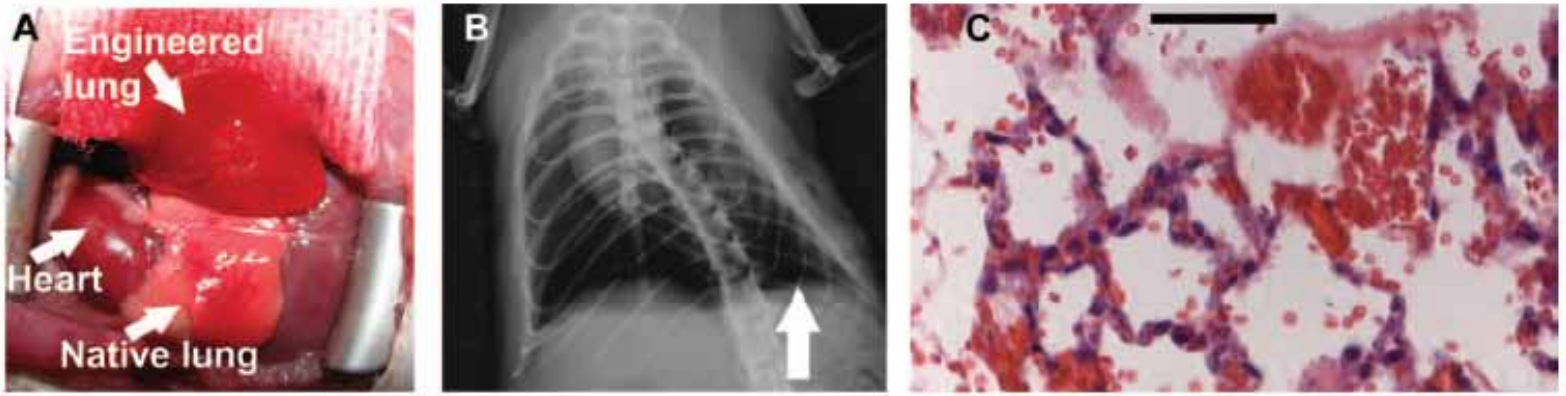

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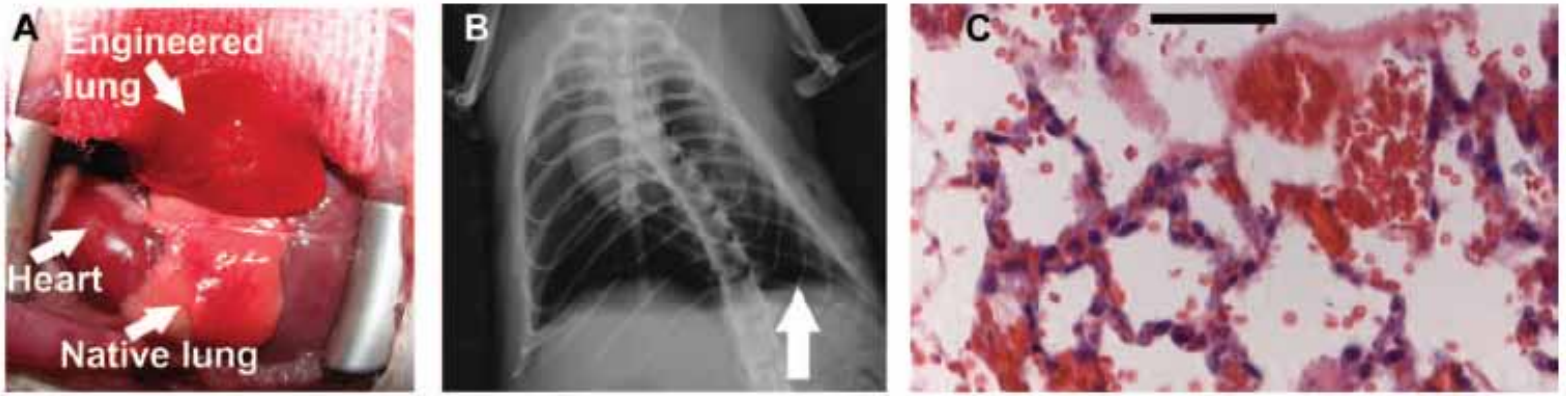

Sample location	pH	Po_2 (mmHg)	O_2 Sat (%)	Pco_2 (mmHg)
Pulmonary artery	7.30 ± 0.06	27 ± 7	44 ± 20	41 ± 13
Right pulmonary vein	7.53 ± 0.08	634 ± 69	100 ± 0	20 ± 1
Left (implant) pulmonary vein	7.68 ± 0.28	283 ± 48	100 ± 0	11 ± 5
Mixed pulmonary veins	7.58 ± 0.08	495 ± 174	100 ± 0	18 ± 3

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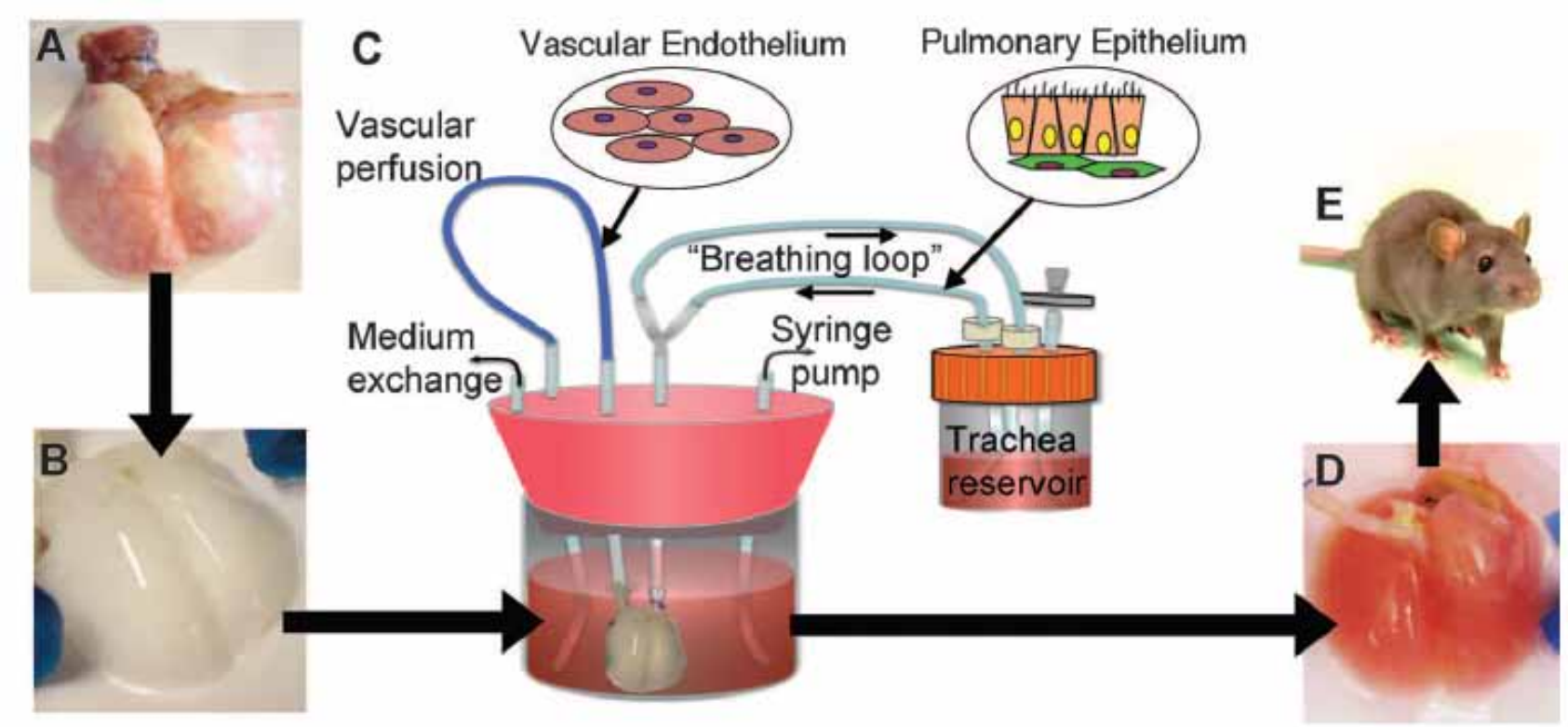
Tissue-Engineered Lungs for in Vivo Implantation



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Tissue-Engineered Lungs for in Vivo Implantation

VI Perspectives

- Autologous source of pulmonary epithelium
  - iPSC
  - Resident lung stem cells
- Improved surfactant production for enhanced compliance
- Enhanced alveolar barrier function
- Enhanced vascular endothelial coverage

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Clinical transplantation of a tissue-engineered airway

Paolo Macchiarini, Philipp Jungebluth, Tetsuhiko Go, M Adelaide Asnaghi, Louisa E Rees, Tristan A Cogan, Amanda Dodson, Jaume Martorell, Silvia Bellini, Pier Paolo Parnigotto, Sally C Dickinson, Anthony P Hollander, Sara Mantero, Maria Teresa Conconi, Martin A Birchall

Vol 372 Decembre 13 2008. The Lancet

Clinical transplantation of a tissue-engineered airway

I Introduction

- Large airway defects are a challenge to clinicians because of the lack of efficient methods of treatment
  - Tracheal resection with primary repair is the only curative treatment
  - Resecable length < 30% in children & <6 cm in adults
  - Previous attempts of autologous or synthetic grafts were disappointing

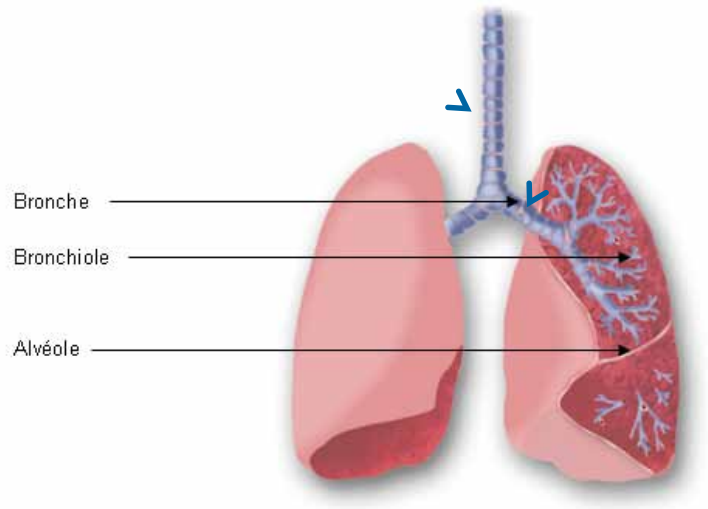
- The aim of this study is to assess the use of a reseeded decellularized tracheal scaffold for the treatment of end-stage airway disease

Paolo Macchiarini *et al*. Vol 372 Decembre 13 2008. The Lancet

Clinical transplantation of a tissue-engineered airway

II Medical History

- A 30 year old woman presented in 2004
  - Cough and dysphonia
  - Due to tuberculous infiltration of cervical trachea and entire left main bronchus



Paolo Macchiarini *et al*. Vol 372 Decembre 13 2008. The Lancet

Clinical transplantation of a tissue-engineered airway

II Medical History

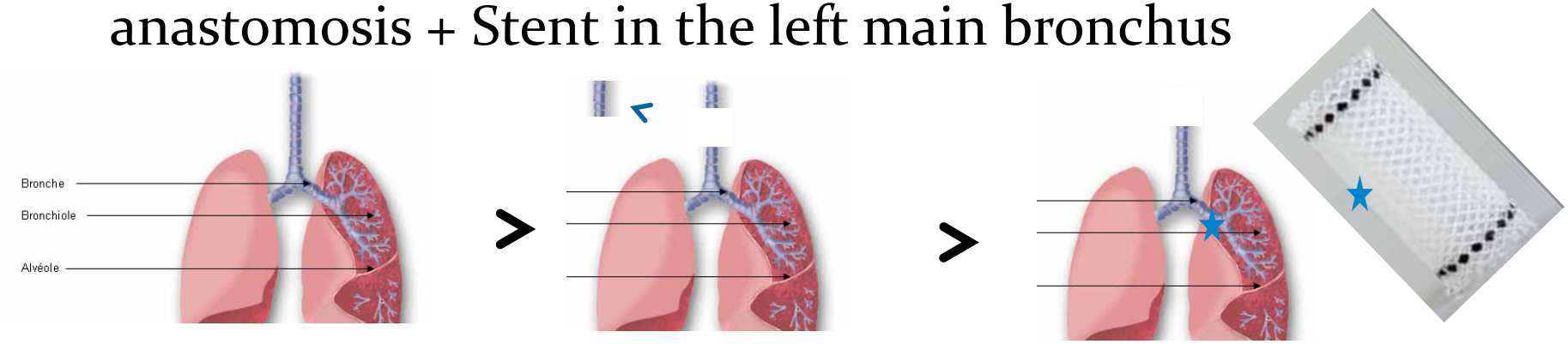
- A 30 year old woman presented in 2004
  - Cough and dysphonia
  - Due to tuberculous infiltration of cervical trachea and entire left main bronchus
  - A CT scan showed a circumferential, near-occlusive 3 cm airway stenosis starting 2cm subglotically and a hypoplasic left main bronchus with expiratory collapse
  - The infection was successfully treated in 6 months

Paolo Macchiarini *et al*. Vol 372 Decembre 13 2008. The Lancet

Clinical transplantation of a tissue-engineered airway

II Medical History

- Severe dyspnea presisted
  - Histology: squamous metaplasia without residual infection
  - Diag: Post-tuberculosis chronic tracheitis and secondary severe bronchomalacia of left main bronchus
  - TT: Subglottic tracheal resection/end-to-end anastomosis + Stent in the left main bronchus



Paolo Macchiarini *et al*. Vol 372 Decembre 13 2008. The Lancet

Clinical transplantation of a tissue-engineered airway

II Medical History

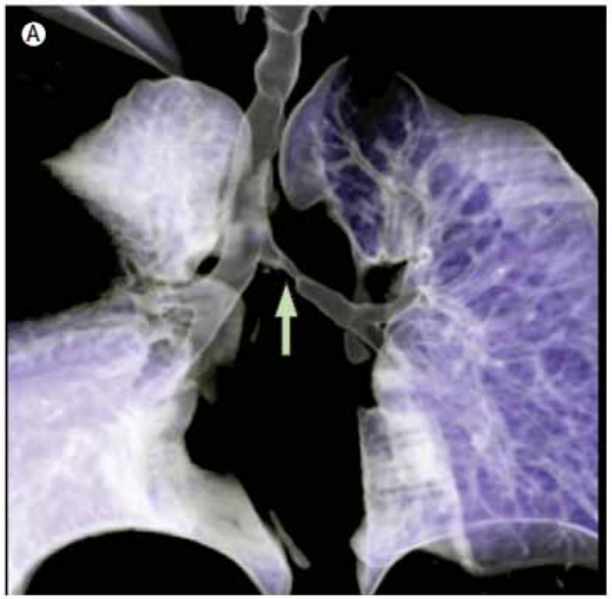
- The stent was poorly tolerated
  - Multiple endoscopic toilets, repositionning and replacement procedures
  - Recurrent episodes of pneumonitis in the left lower lobe
  - Untreatable cough and mucus retention
  - The stent was removed

Paolo Macchiarini *et al*. Vol 372 Decembre 13 2008. The Lancet

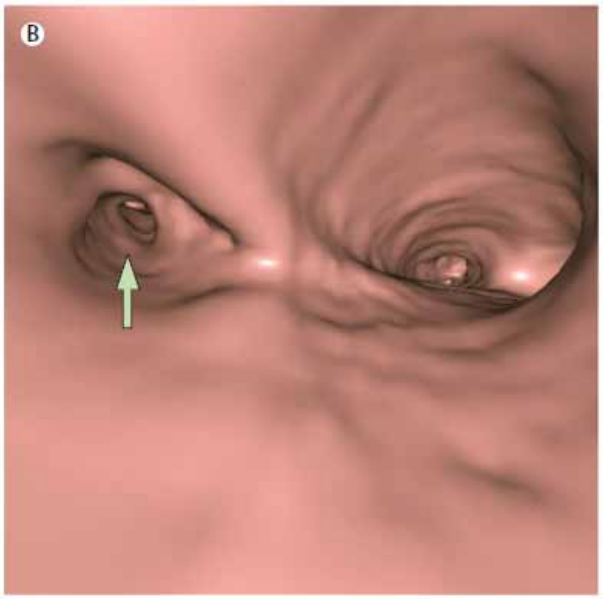
Clinical transplantation of a tissue-engineered airway

II Medical History

- In march 2008, the patient, age 34, was admitted for severe dyspnea (stage III of NYHA classification)



Volume rendering CT-Scan



Virtual bronchoscopy

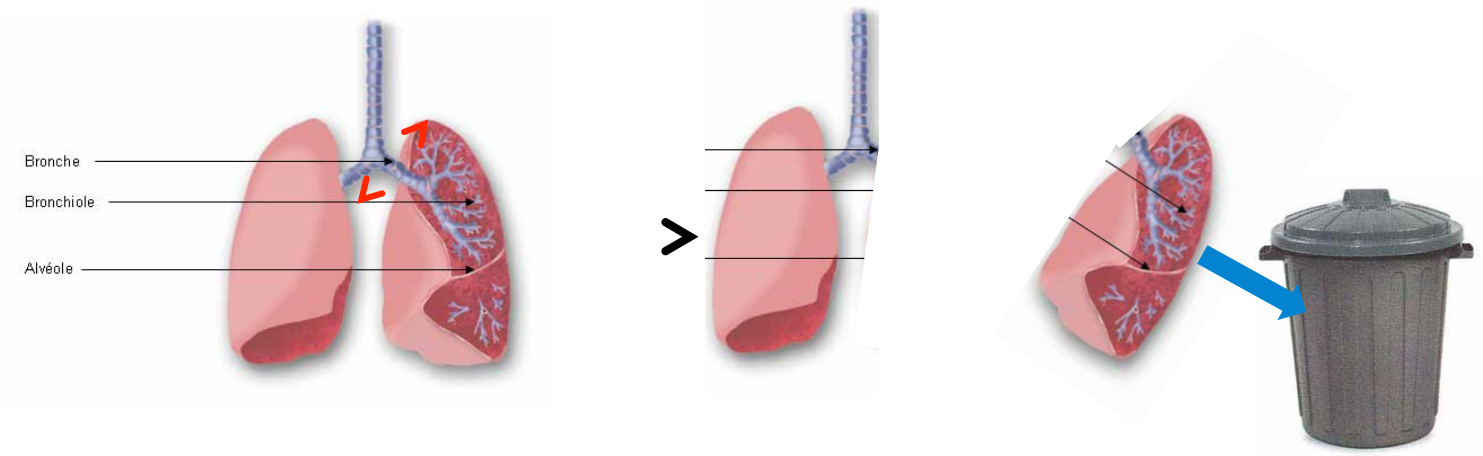
Paolo Macchiarini *et al*. Vol 372 Decembre 13 2008. The Lancet

Clinical transplantation of a tissue-engineered airway

II Medical History

- In march 2008, the patient, age 34, was admitted for severe dyspnea (stage III of NYHA classification)

- The only remaining conventional option was
  - left carinal total pneumonectomy



Paolo Macchiarini *et al*. Vol 372 Decembre 13 2008. The Lancet

Clinical transplantation of a tissue-engineered airway

III Strategy

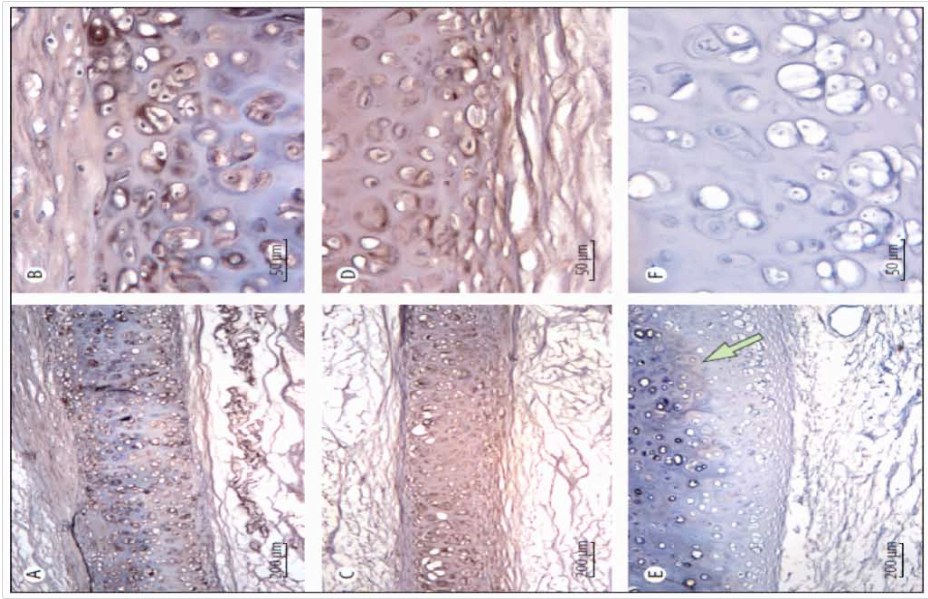
- The authors proposed left main bronchus resection and replacement with a bioengineered human trachea

Paolo Macchiarini *et al*. Vol 372 Decembre 13 2008. The Lancet

Clinical transplantation of a tissue-engineered airway

IV Graft Preparation

- Preparation of airway matrix
  - 7 cm tracheal segment
  - 25 cycles of decellularization protocol



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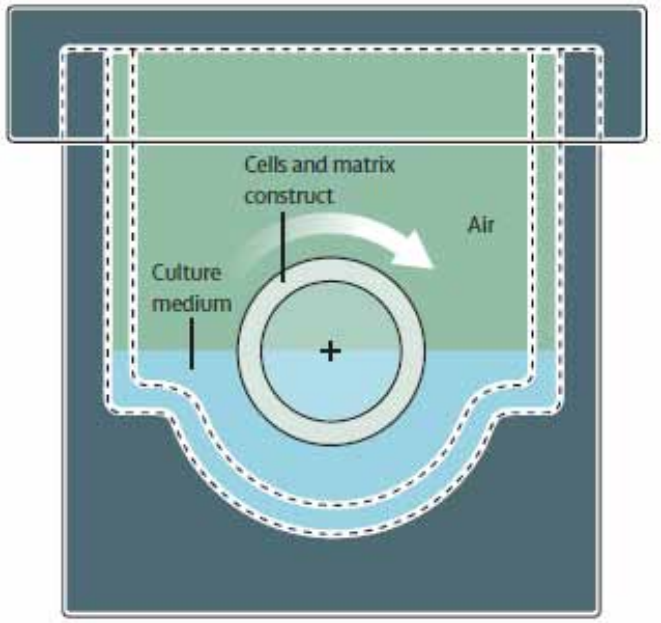
Clinical transplantation of a tissue-engineered airway

IV Graft Preparation

- Preparation of airway matrix
- Bioreactor design



Paolo Macchiarini *et al*. Vol 372 Decembre 13 2008. The Lancet

Clinical transplantation of a tissue-engineered airway

IV Graft Preparation

- Preparation of airway matrix
- Bioreactor design
- Seeding
  - Internal surface: autologous epithelial cells
    - Isolated and grown from bronchoscic biopsy samples
  - External surface: autologuous chondrocytes
    - Induced **differentiation** of mesenchymal stem cells from bone-marrow aspirate

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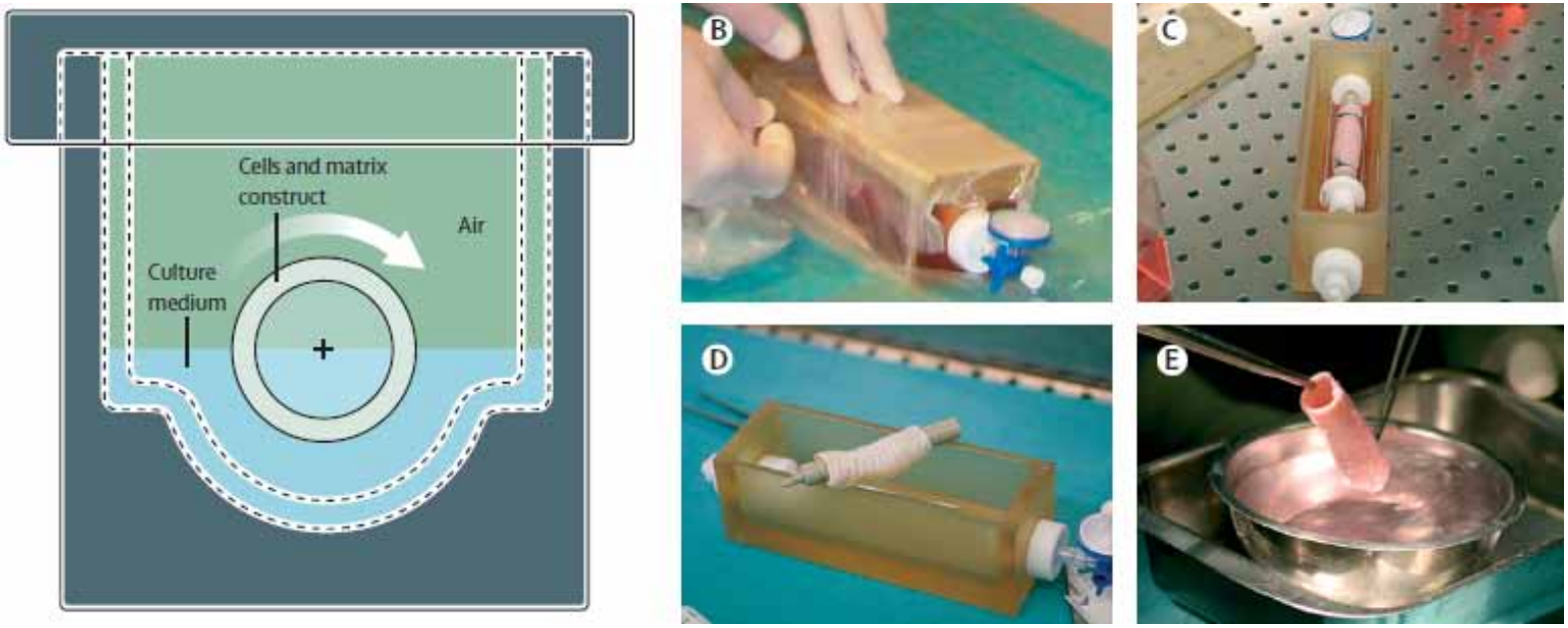
Clinical transplantation of a tissue-engineered airway

IV Graft Preparation

- Preparation of airway matrix
- Bioreactor design
- Seeding



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Clinical transplantation of a tissue-engineered airway

V Graft Implantation and Results

- Evolution was rapidly favorable
  - Improved quality of life
  - Improved Lung Function tests

	December, 2007 (before surgery)	August, 2008 (2 months after surgery)	September, 2008 (3 months after surgery)
FVC (L)	2·35 (62%)	3·81 (100%)	3·86 (100%)
FEV_1 (L)	1·75 (55%)	3·17 (100%)	3·25 (100%)
FEV_1/FVC	0·74	0·95	0·99
Raw (kPa/Lxs)	5·57	3·06	3·31
SGaw ($kPa^{-1}s^{-1}$)	0·058	0·122	0·213

A substantial reduction in forced expiratory volume in 1 s (FEV_1) and a smaller but significant reduction in forced vital capacity (FVC) occurred preoperatively. A complete reversal of airway obstruction, a substantial amelioration in airway resistance and conductance occurred postoperatively. Ratio of FEV_1 to FVC in healthy adults is 0·75–0·80; Raw=airway resistance. SGaw=specific airways conductance.

Table: **Lung function test results**

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Clinical transplantation of a tissue-engineered airway

V Graft Implantation and Results

- Evolution was rapidly favorable
  - Improved quality of life
  - Improved Lung Function tests
  - Endoscopic monitoring of the graft
    - Normal mucosa secretion
    - No inflamation
    - Laser Doppler: rich microvascular bed

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Clinical transplantation of a tissue-engineered airway

V Graft Implantation and Results

- Evolution was rapidly favorable
  - Improved quality of life
  - Improved Lung Function tests
  - Endoscopic monitoring of the graft
  - CT-reconstruction at 1 month post-op
    - Wide potency

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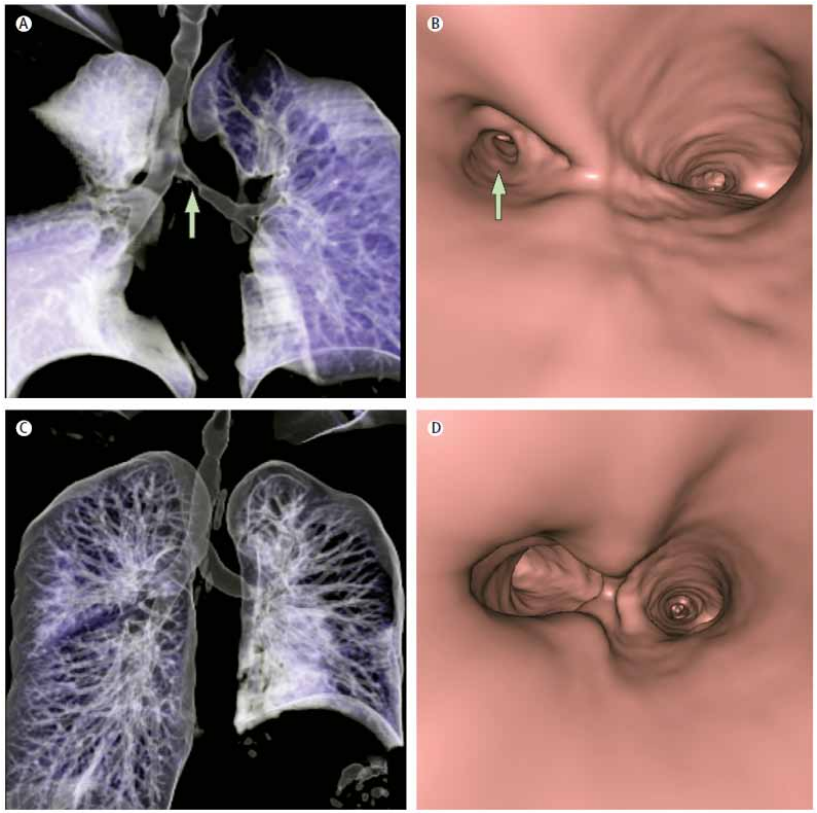
Clinical transplantation of a tissue-engineered airway

V Graft Implantation and Results



CT-reconstruction:
stenosis

CT-reconstruction
at 1 month post-op

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Clinical transplantation of a tissue-engineered airway

V Graft Implantation and Results

- Evolution was rapidely favorable
  - Improved quality of life
  - Improved Lung Function tests
  - Endoscopic monitoring of the graft
  - CT-reconstruction at 1 month post-op
  - Serology
    - Absence of anti-donor HLA antibodies at 14 days, 1 & 2 months

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Clinical transplantation of a tissue-engineered airway

VI Conclusion & Perspectives

- Successful tissue-engineered large airway replacement
  - Improved quality of life & respiratory function
  - No immunosuppressive therapy needed
  - 4 month follow-up

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Clinical transplantation of a tissue-engineered airway

VI Conclusion & Perspectives

- Successful tissue-engineered large airway replacement

- Clinical trial

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Clinical transplantation of a tissue-engineered airway

VI Conclusion & Perspectives

- Successful tissue-engineered large airway replacement
- Clinical trial
- Feasibility of tissue engineering based on the combination of
  - Autologous cells
  - Decellularized tissue matrix

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