BARCELONA RESPIRATORY NETWORK
Collaborative research

Bioengineering Approaches to Lung Repair and Regeneration

Ramon Farré, Ph.D.

Biophysics & Bioengineering Lab
School of Medicine & Health Sciencies
University of Barcelona

UNIVERSITAT DE BARCELONA

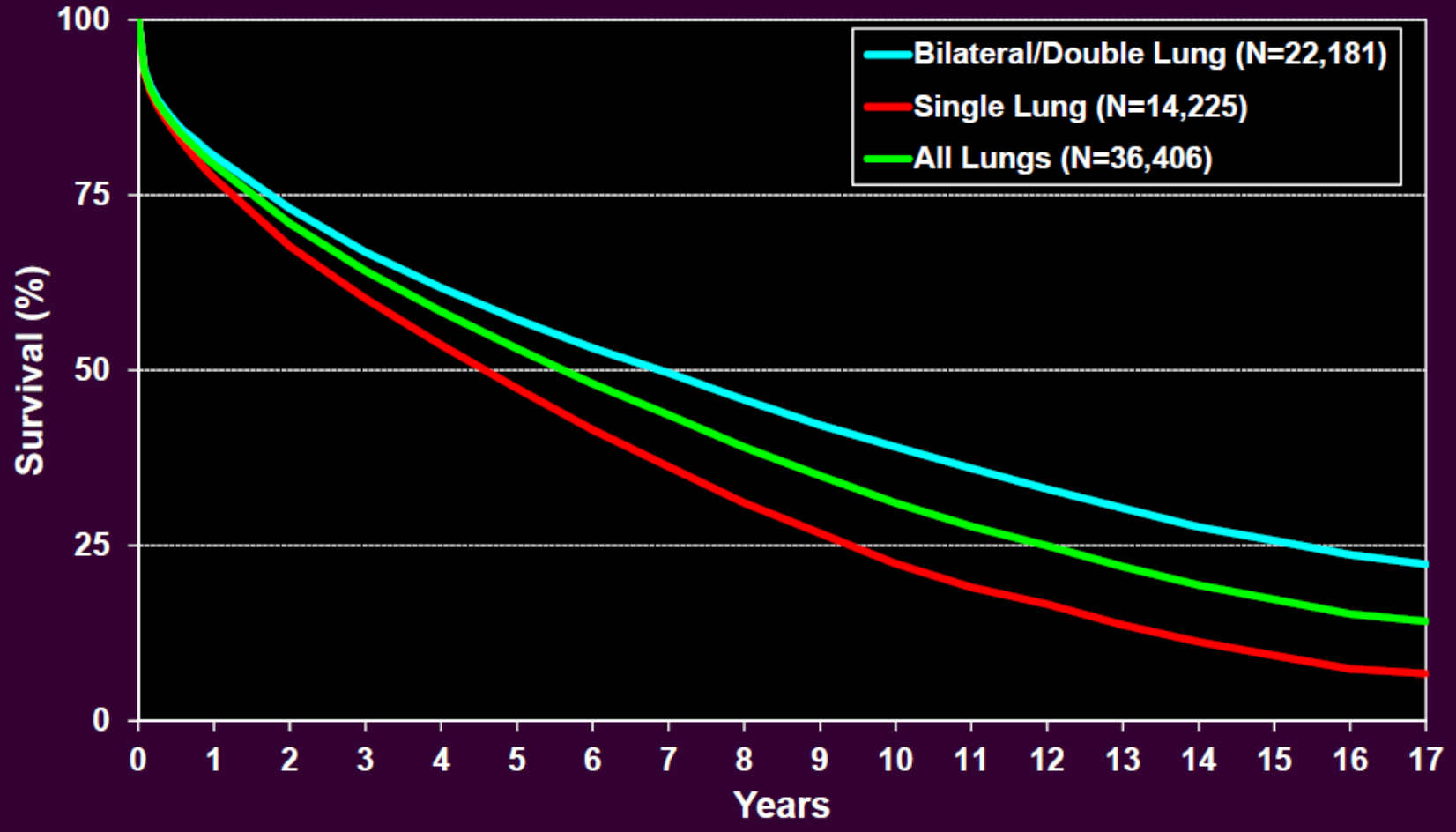
IDIBAPS

ciberes
Centro de Investigación Biomédica en Red
Enfermedades Respiratorias

Adult Lung Transplants

Kaplan-Meier Survival by Procedure Type

(Transplants: January 1994 – June 2011)

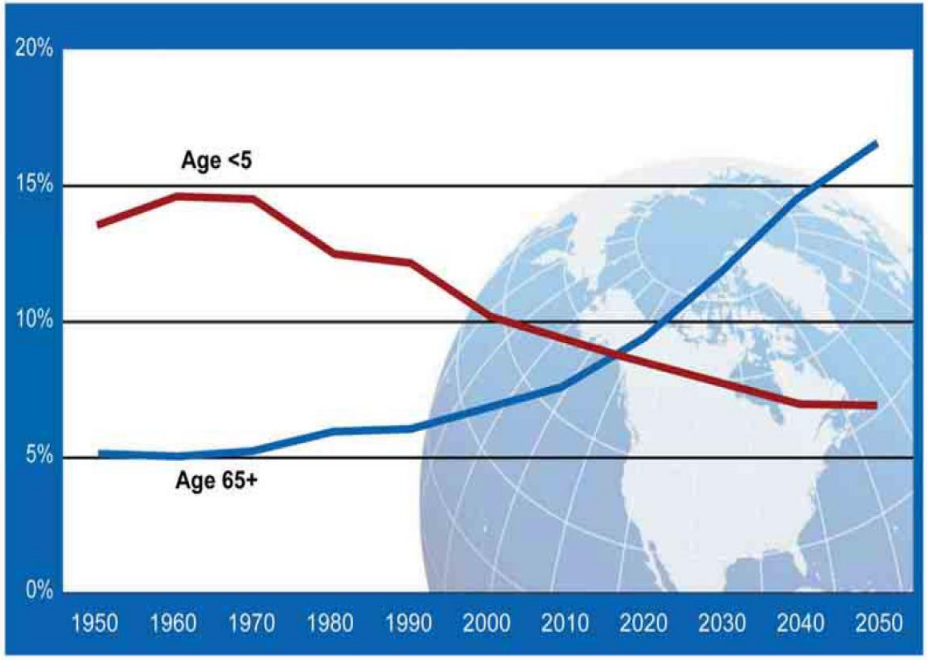


ISHLT • INTERNATIONAL SOCIETY FOR HEART AND LUNG TRANSPLANTATION 2013

JHLT. 2013 Oct; 32(10): 965-978

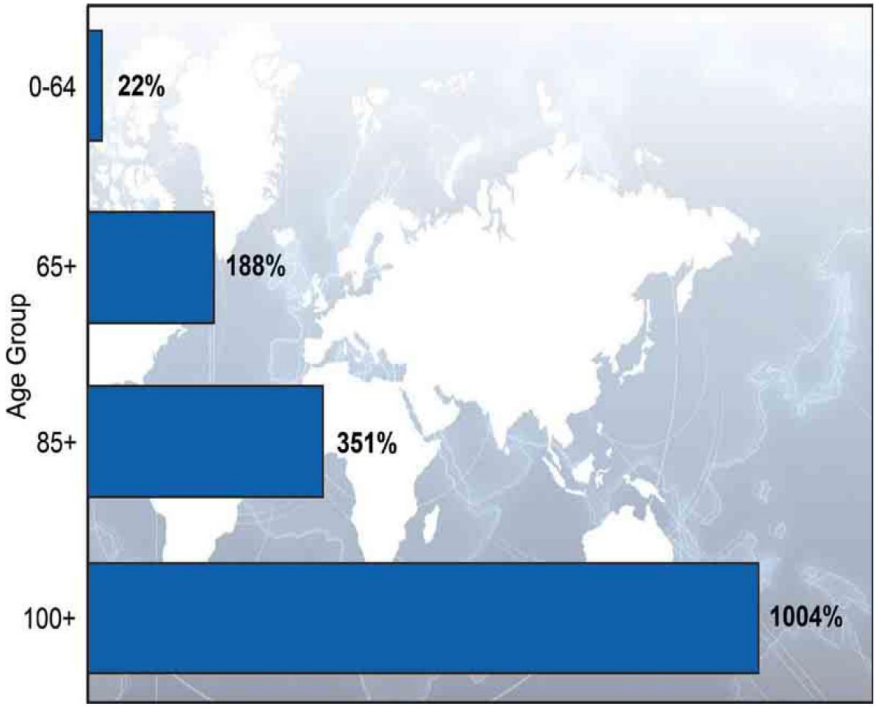
Ageing of the population

Young Children and Older People as a Percentage of Global Population: 1950-2050



Source: United Nations. *World Population Prospects: The 2010 Revision.*

Percentage Change in the World's Population by Age: 2010-2050



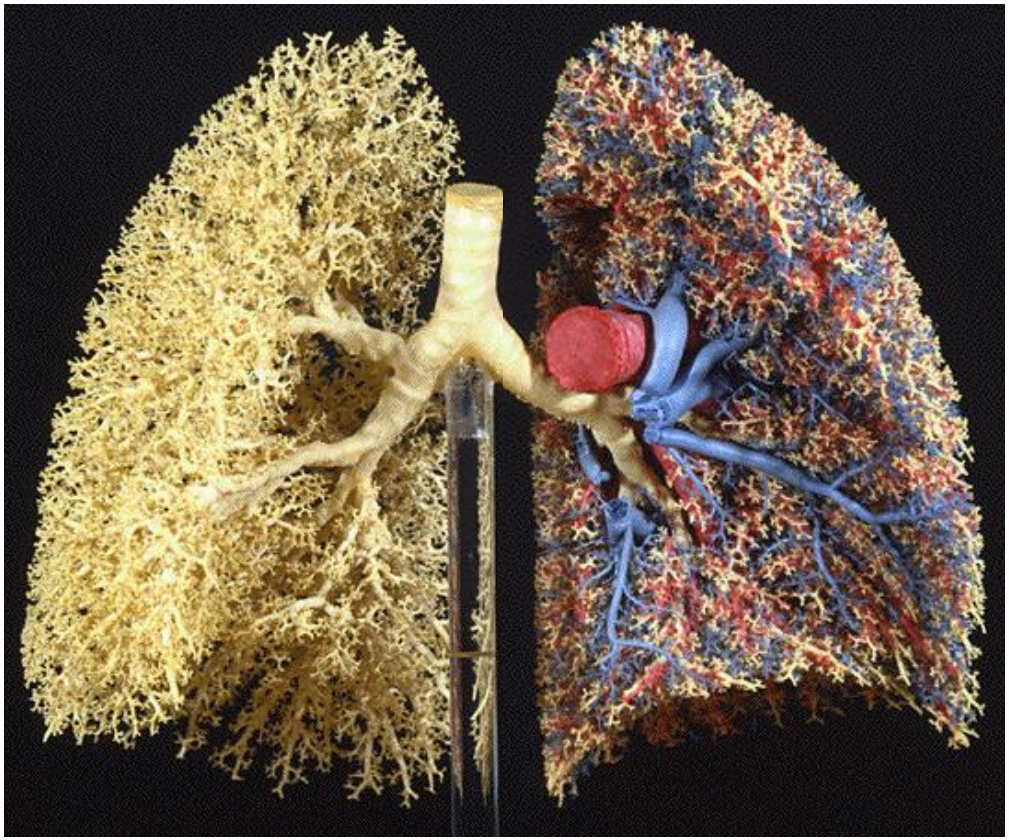
Source: United Nations, *World Population Prospects: The 2010 Revision.*

How to increase the availability of viable organs for transplantation?

A potential solution could the **biofabrication of lungs** using the concepts, tools and very recent advances acheived in the fields of:

- ✓ ***Cell therapy***
- ✓ ***Regenerative Medicine***
- ✓ ***Organ and Tissue Engineering***

Structural complexity of the lungs

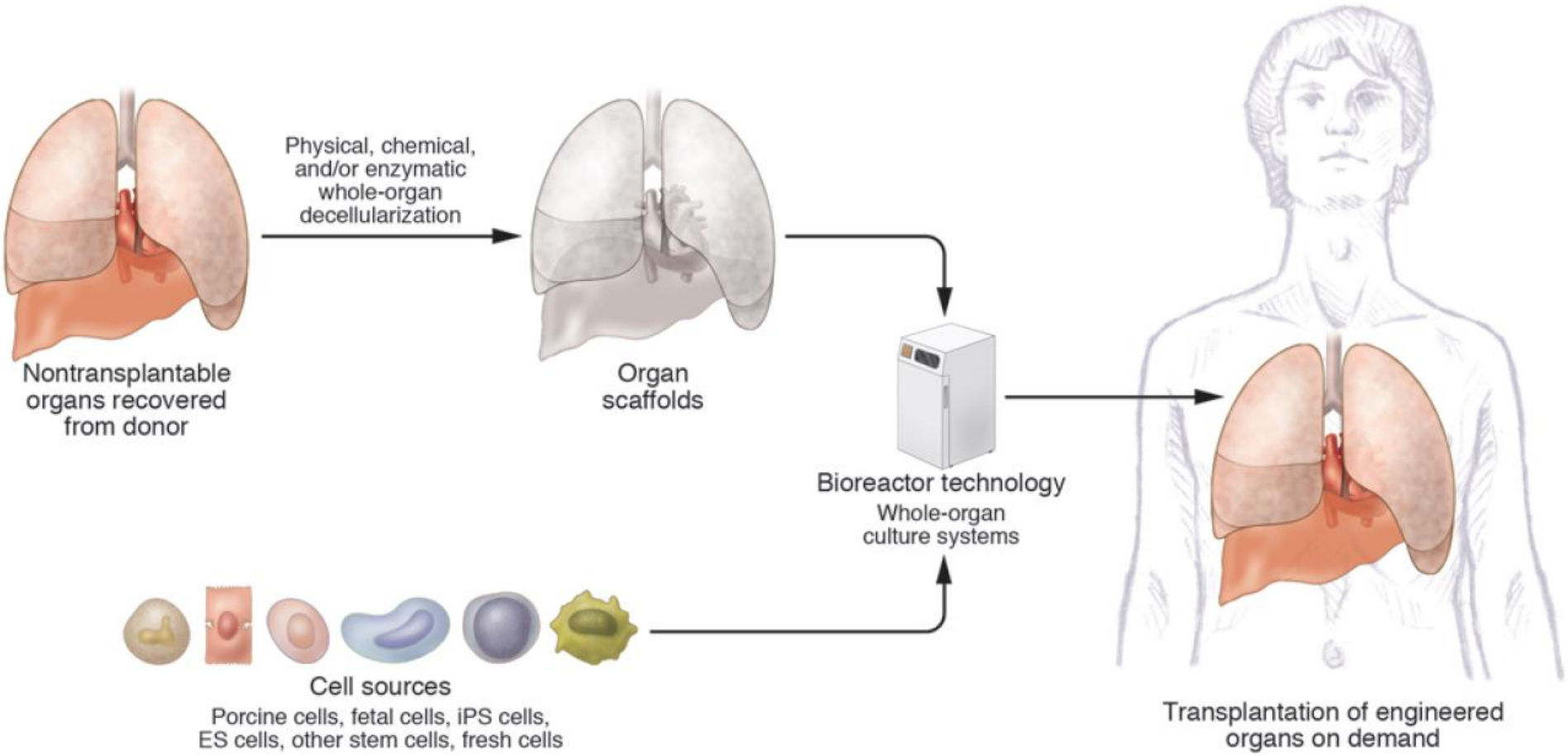


Alveoli: N = 300 millions
d = 0.3 mm

Membrane: S = 70 m^2
w = 0,003 mm

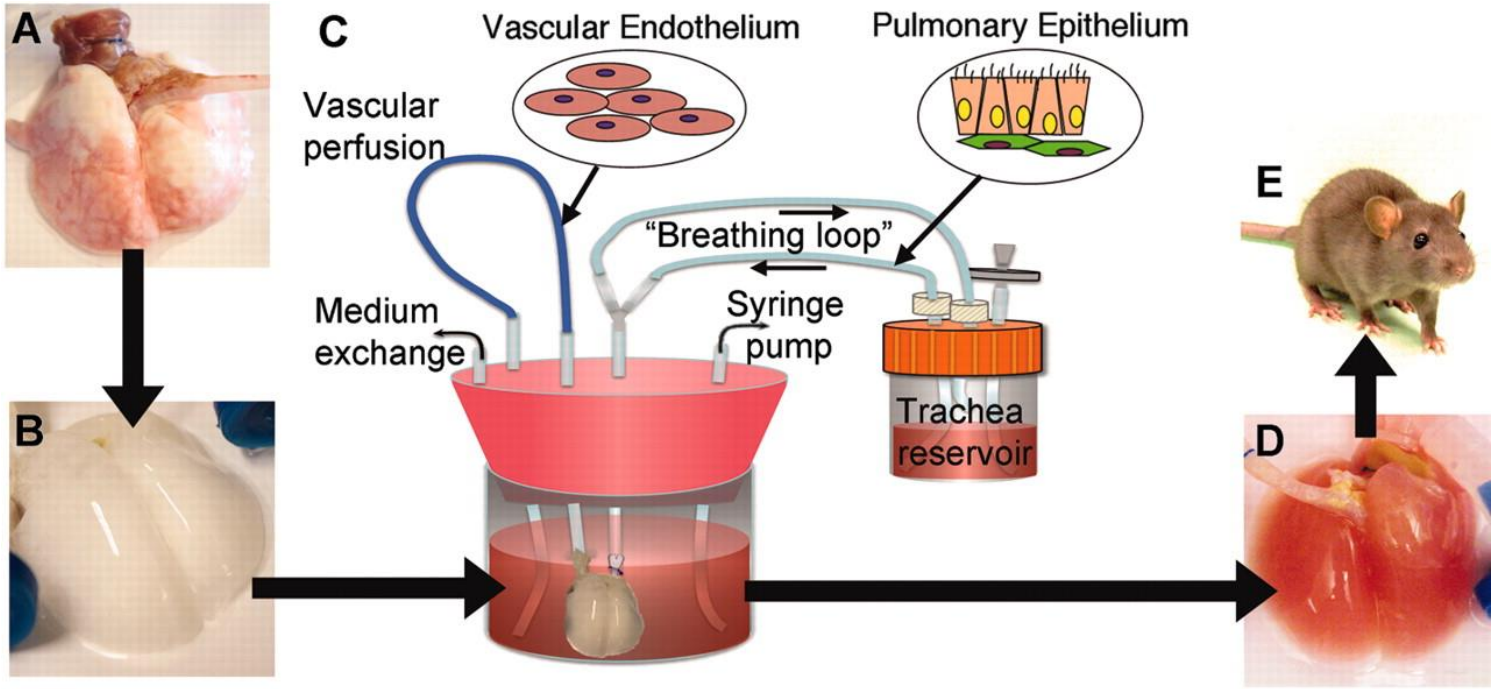
Mechanical stimuli:

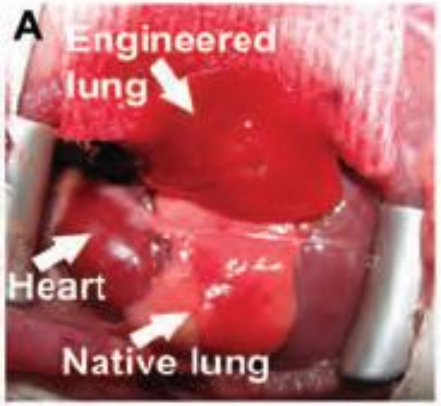
- *Substrate stiffness*
- *Extracellular-matrix tethering*
- *Cell size & shape constriction*
- *3D & 2D environment*



Soto-Gutierrez et al. J Clin Invest. 2012;122(11):3817–3823

Lung bioengineering: proof of concept





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

Petersen *et al. Science* 2010; 329:538-41

The NEW ENGLAND JOURNAL of MEDICINE

EDITORIALS

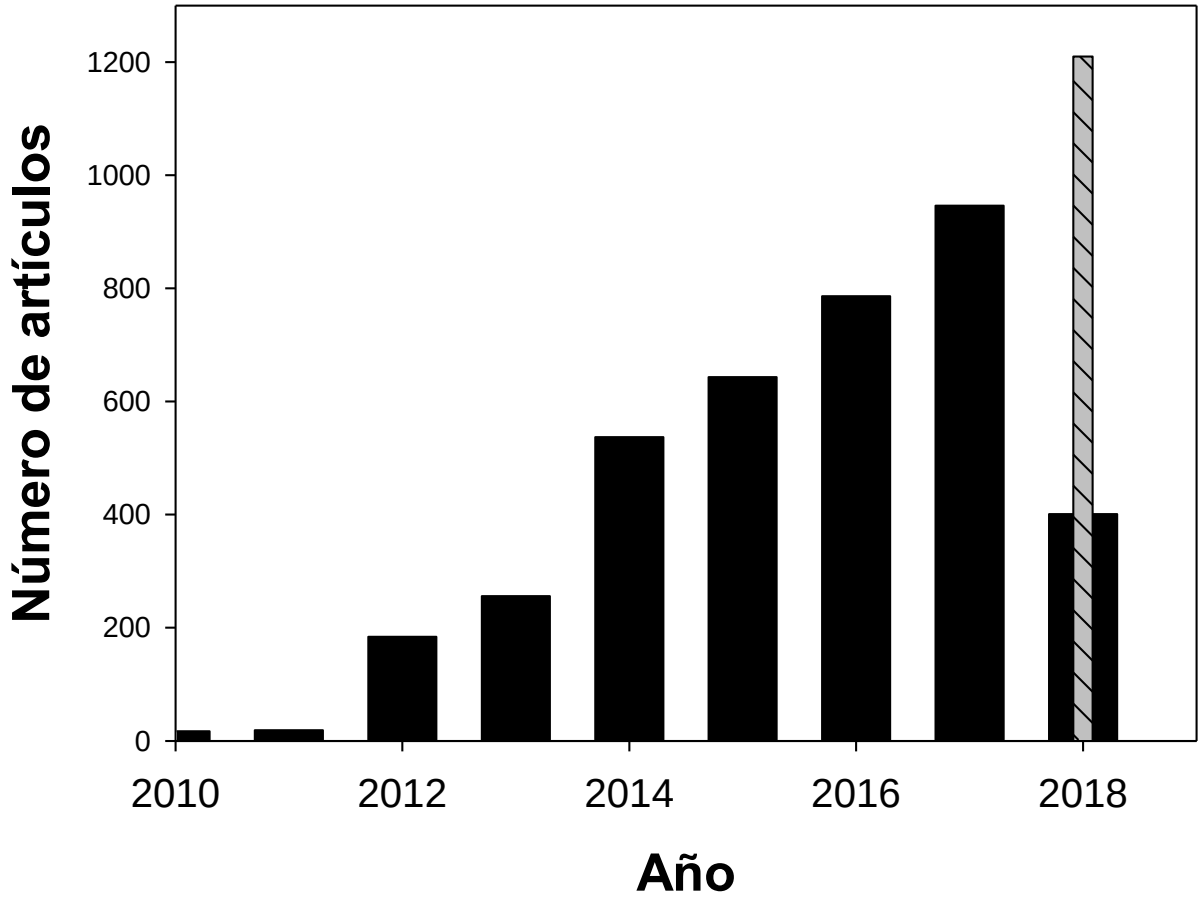
N ENGL J MED 364;19 NEJM.ORG MAY 12, 2011

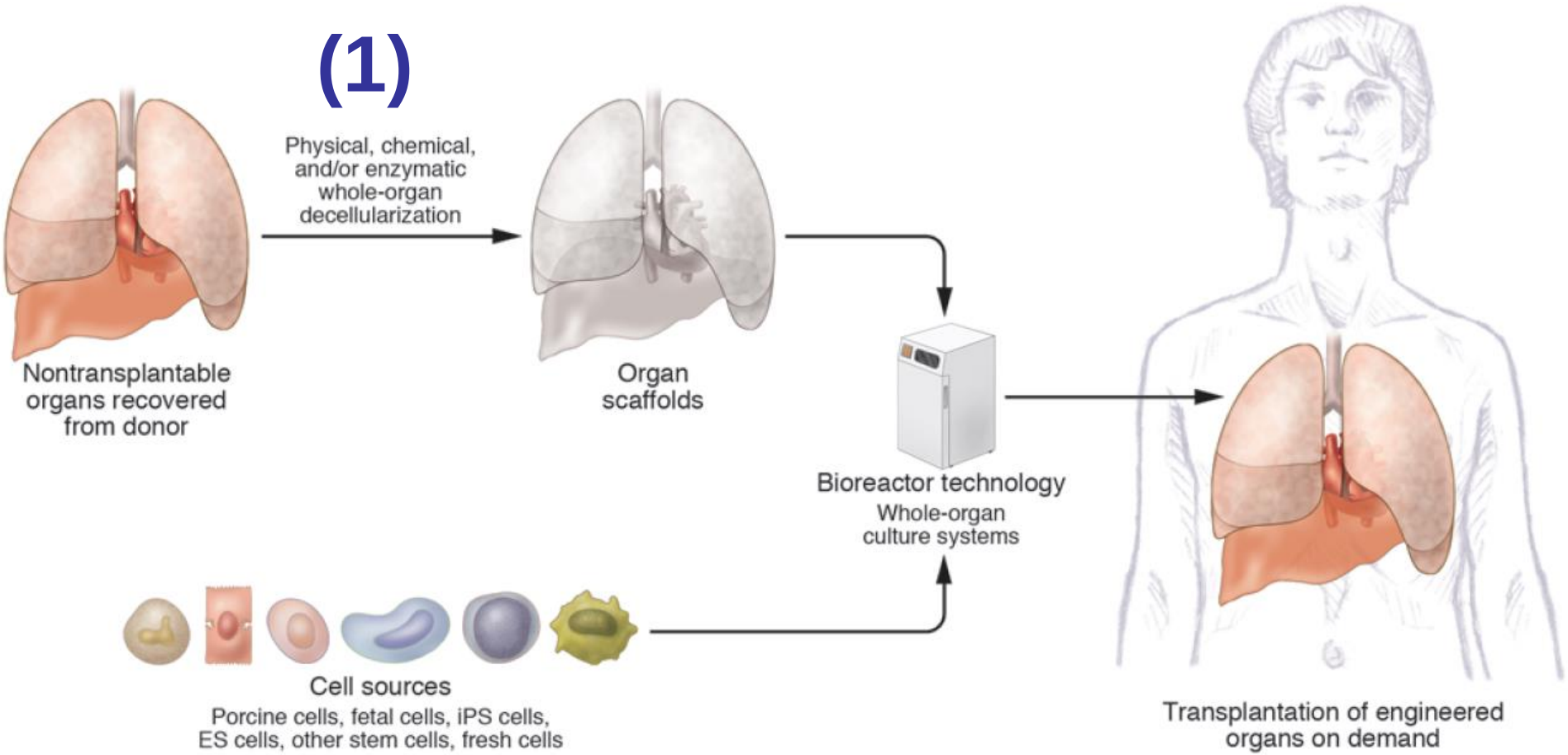
Toward Lung Regeneration

Harold A. Chapman, M.D.

The field of bioengineered lung tissue sees clinical medicine on the distant horizon. But patients are living with airway transplants of devitalized tracheal matrix repopulated with their own epithelial cells.[7] It is possible to grow well-organized rodent lung tissue beginning with an intact, devitalized lung matrix and embryonic lung cells and to observe a functional effect, if only briefly, when this tissue is implanted in live animals.[8]

PubMed: "lung AND bioengineering"



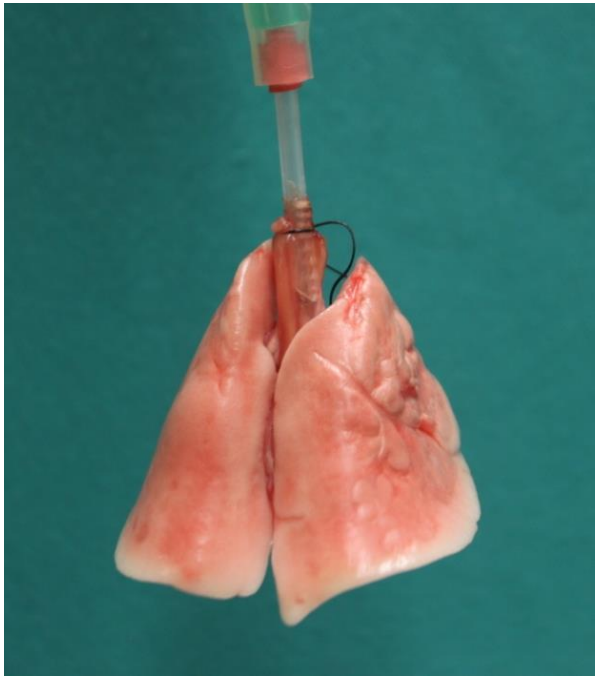


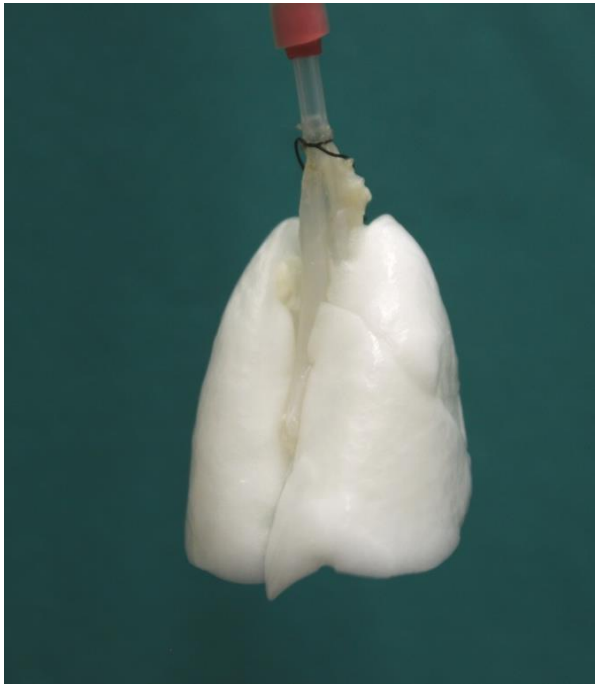
Soto-Gutierrez et al. J Clin Invest. 2012;122(11):3817–3823

LUNG DECELLULARIZATION

- *Full elimination of donor cell material*

- *Preservation of extracellular matrix proteins*





Decellularization of tissues and organs

Physical	Enzymatic	Chemical
Mechanical agitation	Trypsin	Alkaline/acid
		Hypotonic and hypertonic solutions
Freeze/thaw	Endonucleases	EDTA, EGTA
Sonication	Exonucleases	**~Nonionic detergents**
		Triton X-100
		~Ionic detergents
		Sodium dodecyl sulfate (SDS)
		Triton X-200
		~Zwitterionic detergents
		CHAPS
		Sulfobetaine-10 and -16 (SB-10, SB-16)
		Tri(n-butyl)phosphate

Badylak et al. Annu. Rev. Biomed. Eng. 2011. 13:27–53

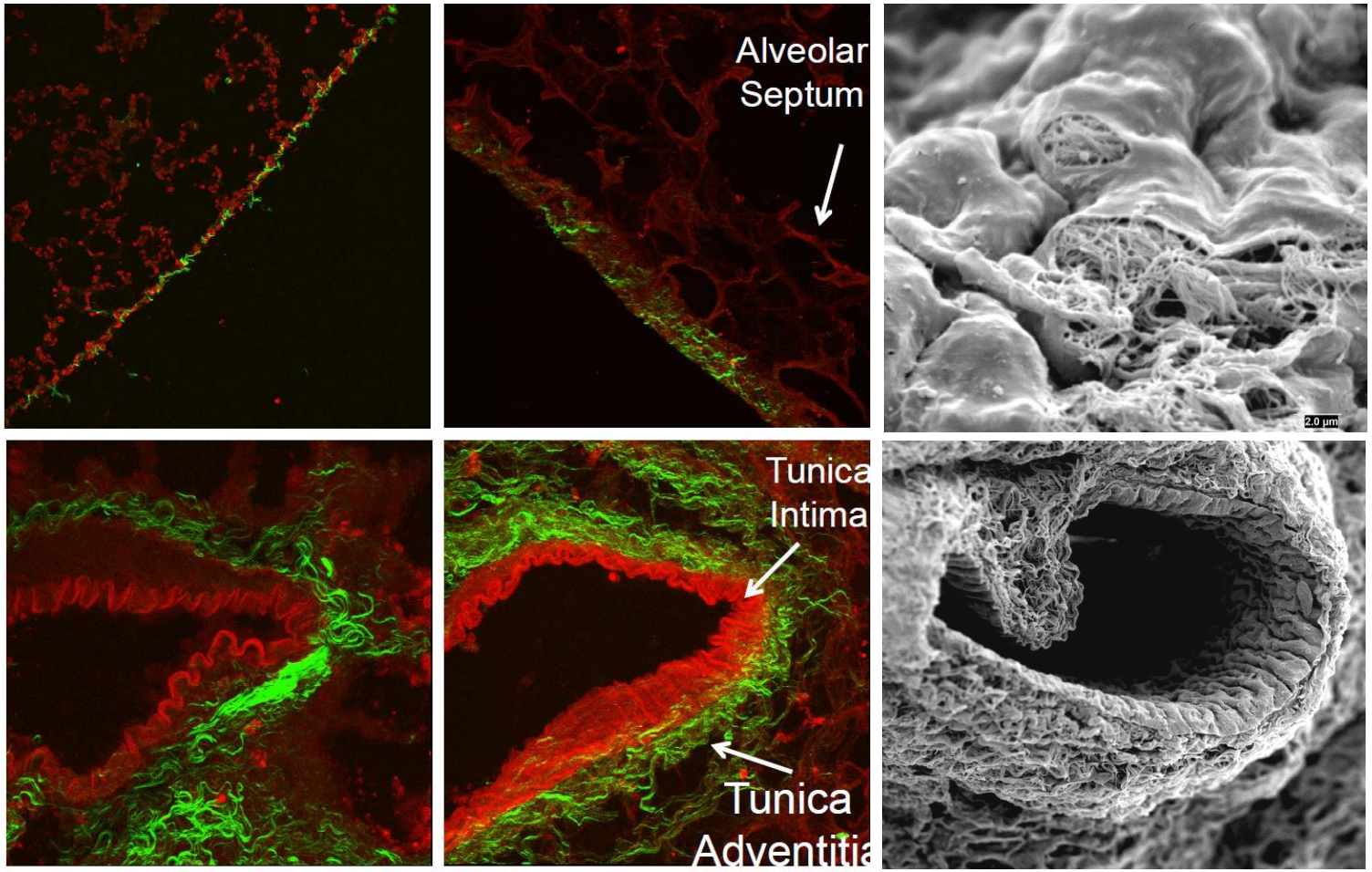
Effects of the Decellularization Method on the Local Stiffness of Acellular Lungs

Esther Melo, BSc,[1–3] Elena Garreta, PhD,[1–3] Tomas Luque, BSc,[1,2,4] Joaquin Cortiella, PhD,[5] Joan Nichols, PhD,[5] Daniel Navajas, PhD,[1,2,4] and Ramon Farré, PhD[1–3]

TISSUE ENGINEERING: Part C
Volume 20, Number 5, 2014

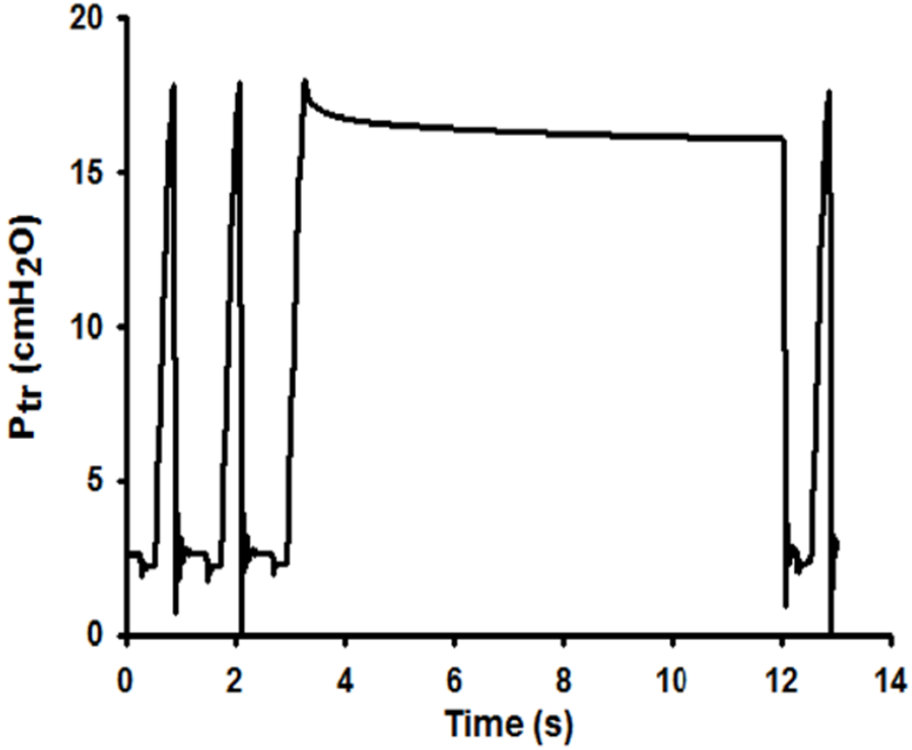
Nativo *Descelularizado*

Colágeno
Elastina



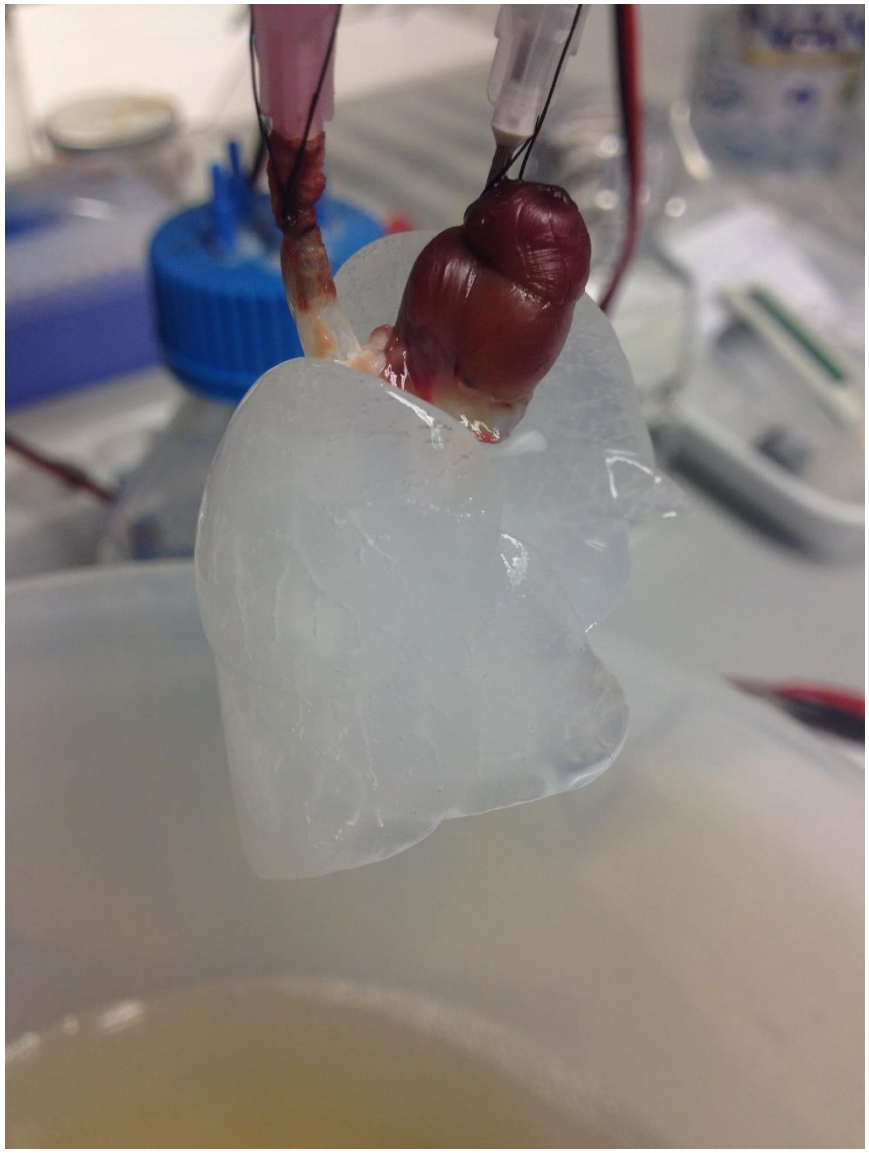
Lung decellularization preserves integrity of:

- airways:

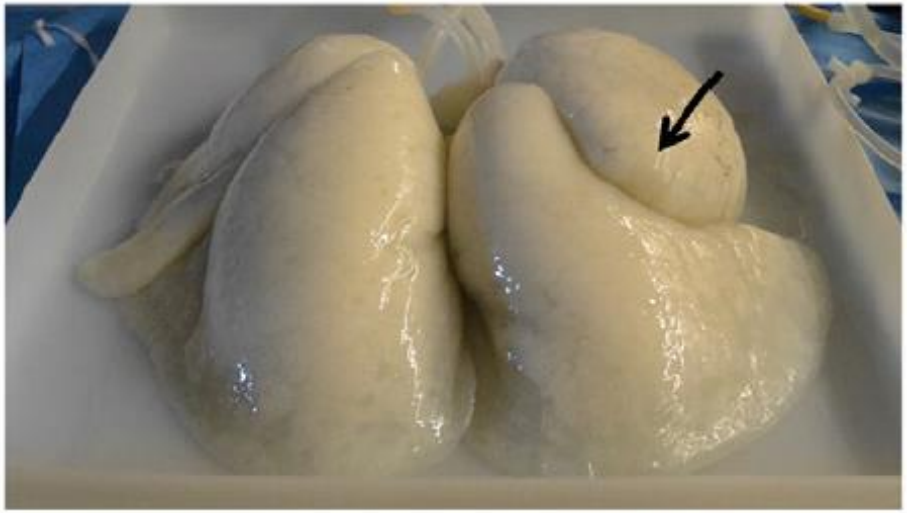


- Vascular circuit:

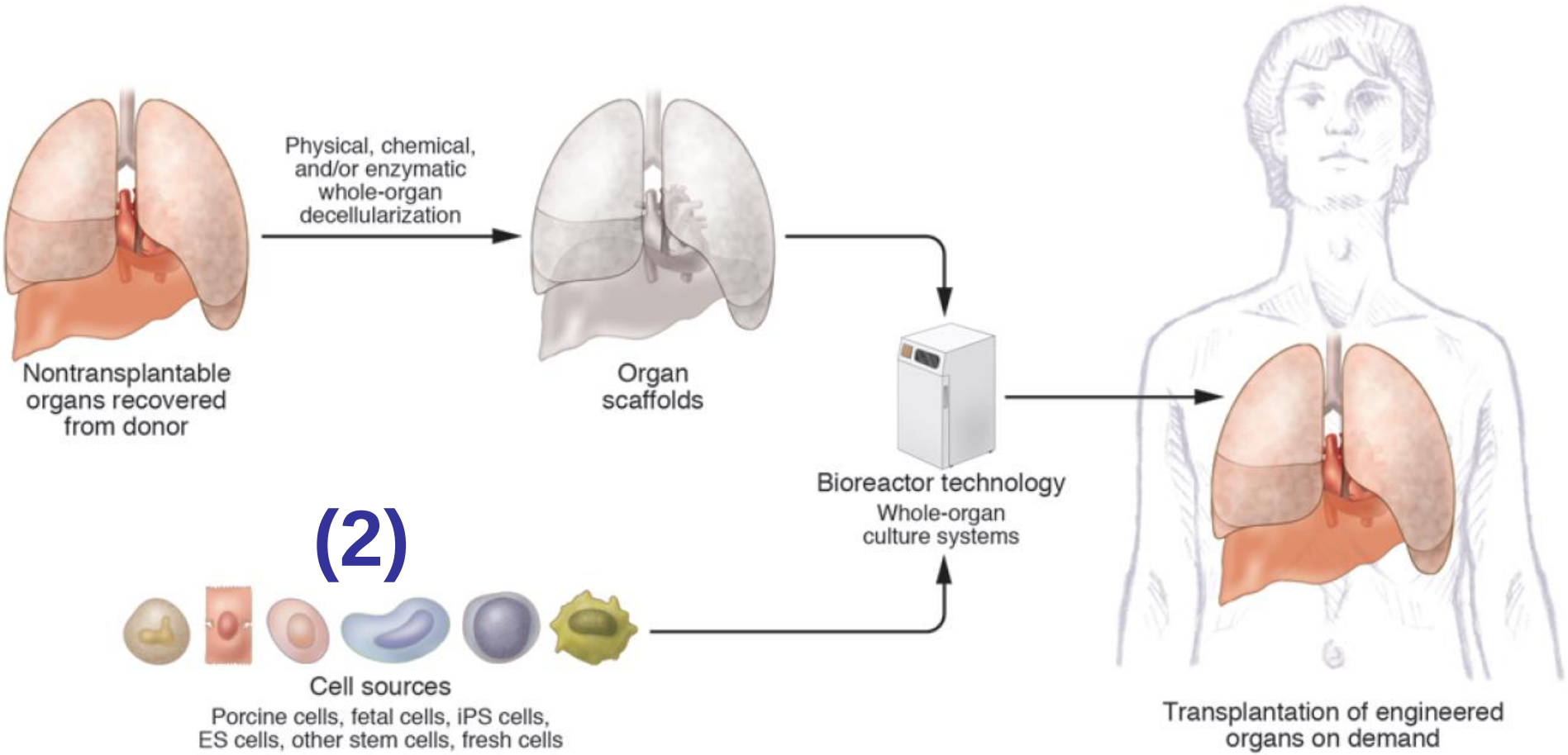
Automatic decellularization



Decellularization of human lungs



Gilpin *et al. J Heart Lung Transplant 33:298–308, 2014*

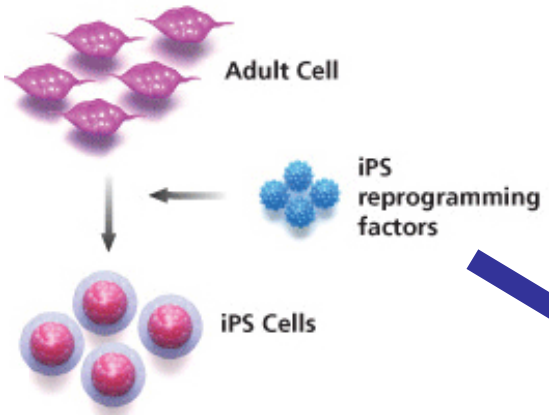


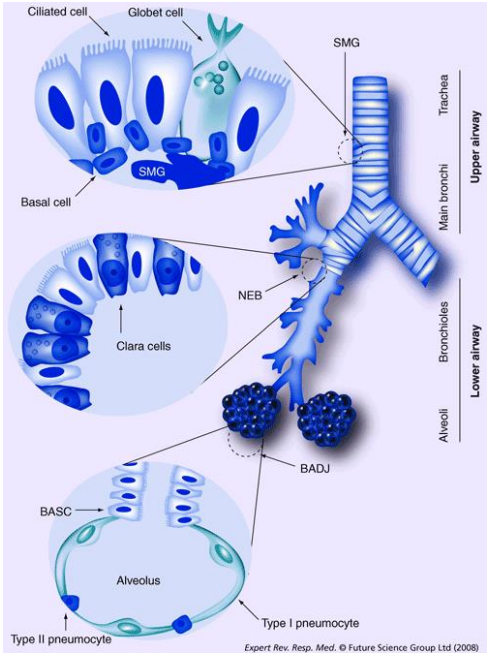
Soto-Gutierrez et al. J Clin Invest. 2012;122(11):3817–3823

RECELLULARIZATION WITH STEM CELLS:

¿Embryonic?

¿Adult?





The NEW ENGLAND JOURNAL of MEDICINE

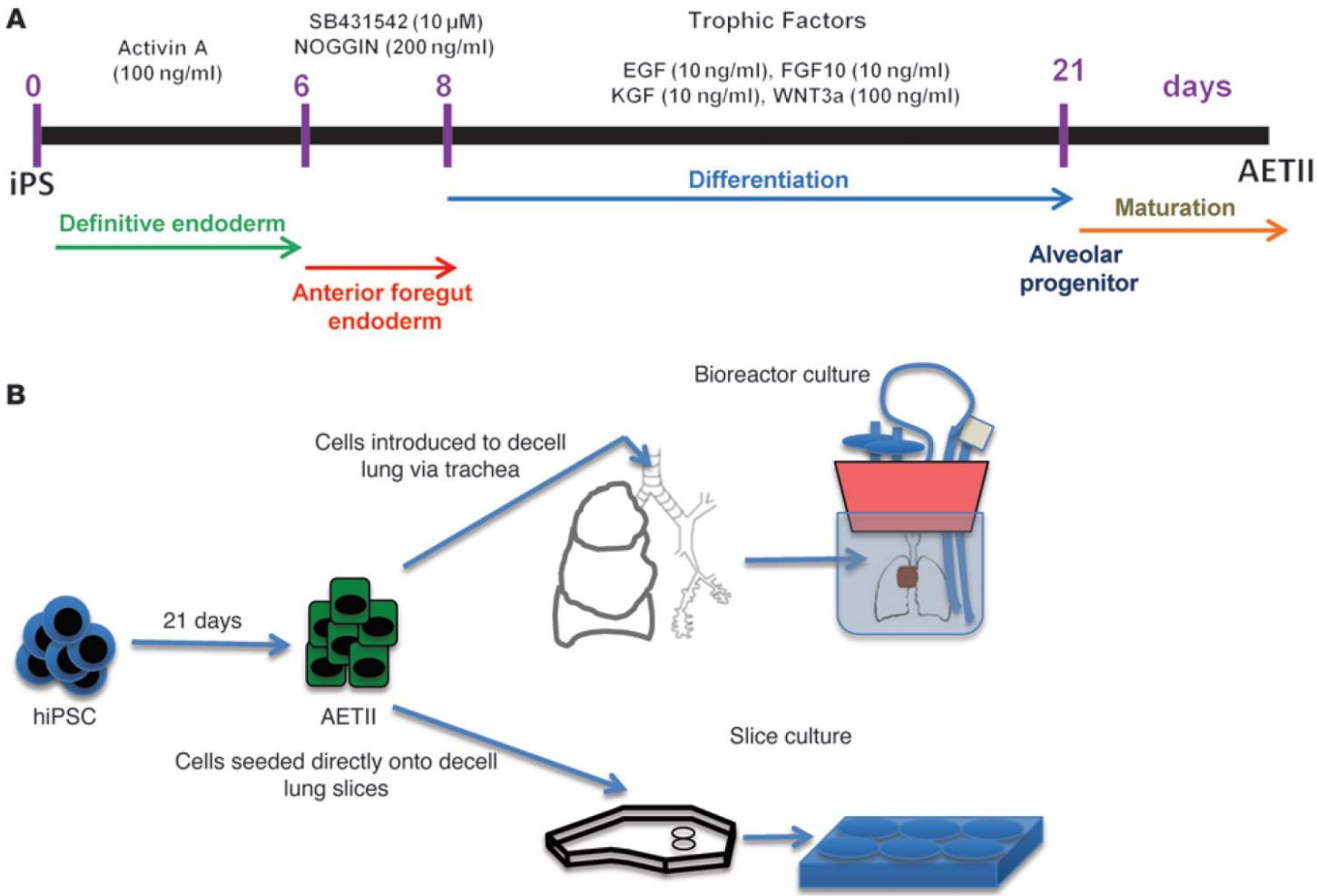
ESTABLISHED IN 1812 MAY 12, 2011 VOL. 364 NO. 19

Evidence for Human Lung Stem Cells

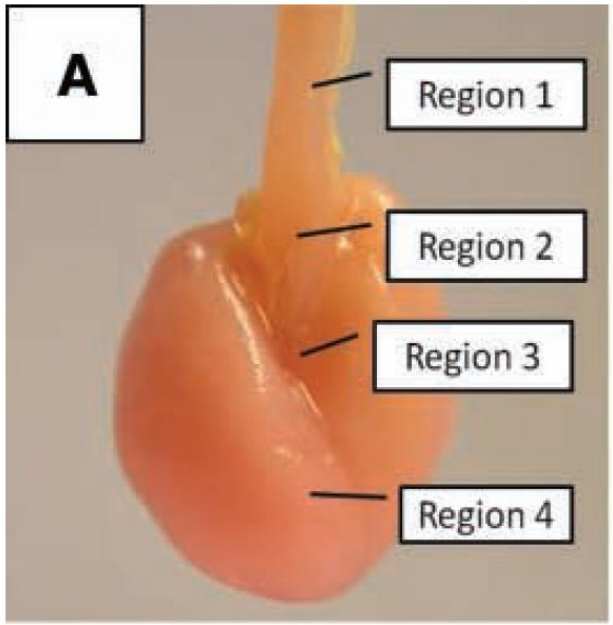
Human iPS cell–derived alveolar epithelium repopulates lung extracellular matrix

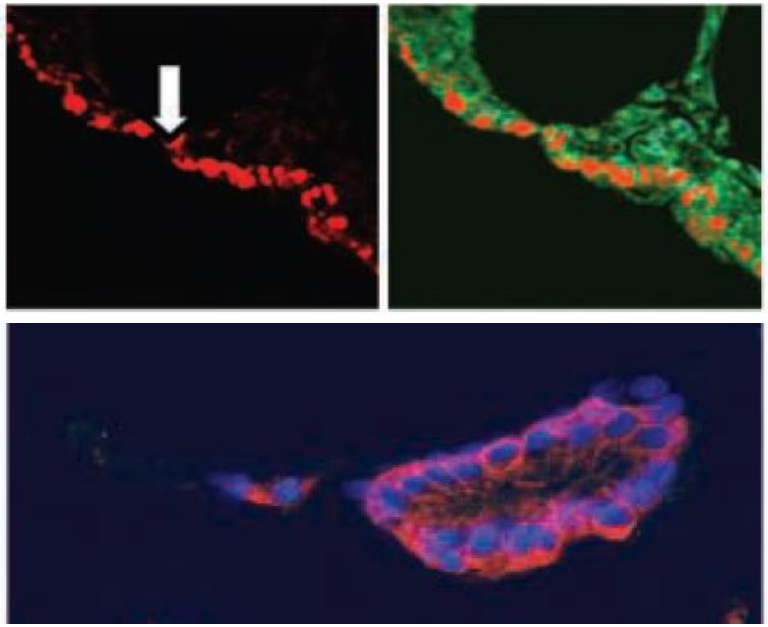
Mahboobe Ghaedi,[1] Elizabeth A. Calle,[1] Julio J. Mendez,[1] Ashley L. Gard,[1] Jenna Balestrini,[1] Adam Booth,[2] Peter F. Bove,[3] Liqiong Gui,[1] Eric S. White,[2] and Laura E. Niklason[1]

J Clin Invest. 2013;123(11):4950–4962.



Las células madre aplicadas a la matriz pulmonar experimentan una diferenciación acorde con la zona pulmonar en que se encuentran.

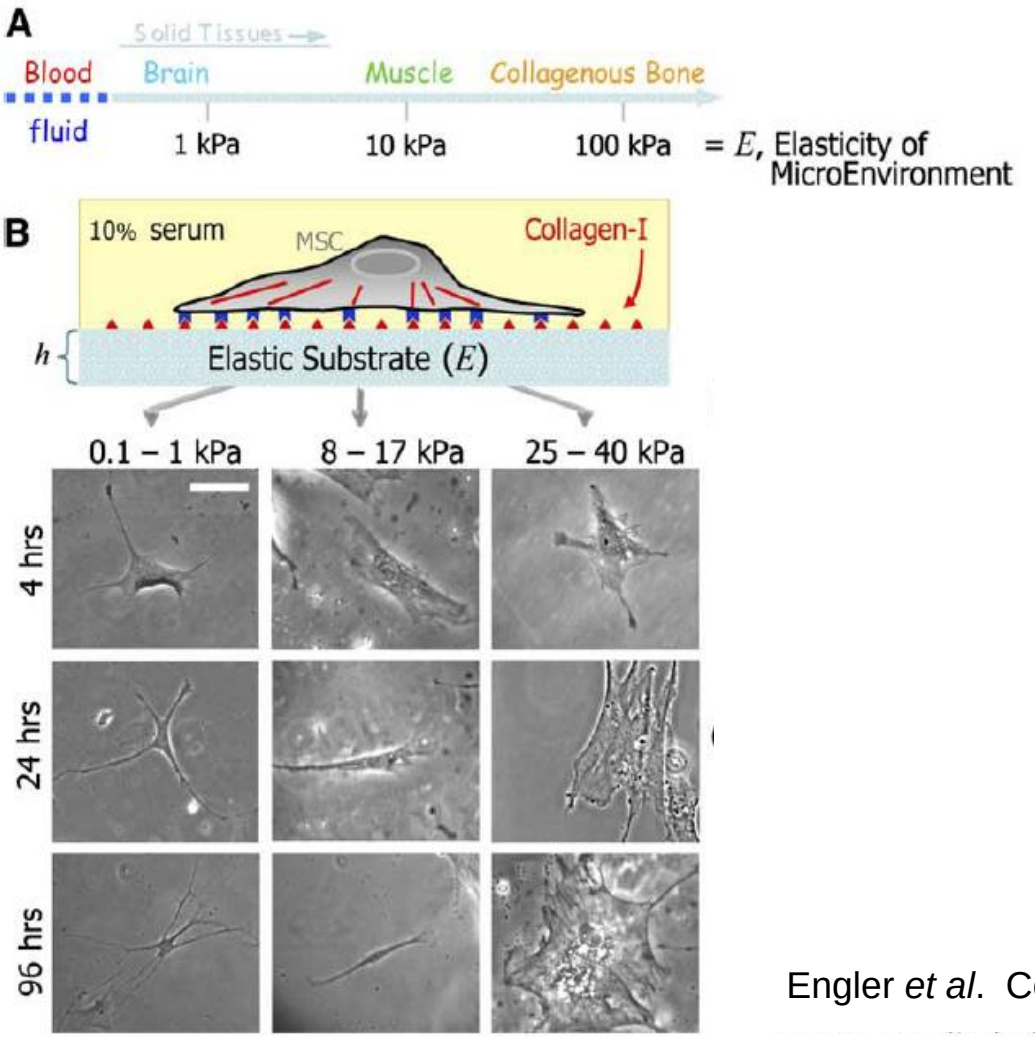




Cortiella *y col*. 2010

El microentorno mecánico de las células madre (rigidez, deformación y estructura tridimensional de la matriz pulmonar) modula la diferenciación hacia los diferentes tipos celulares en el pulmón.

La diferenciación de células madres, tanto embrionarias como adultas, depende de la rigidez del sustrato.



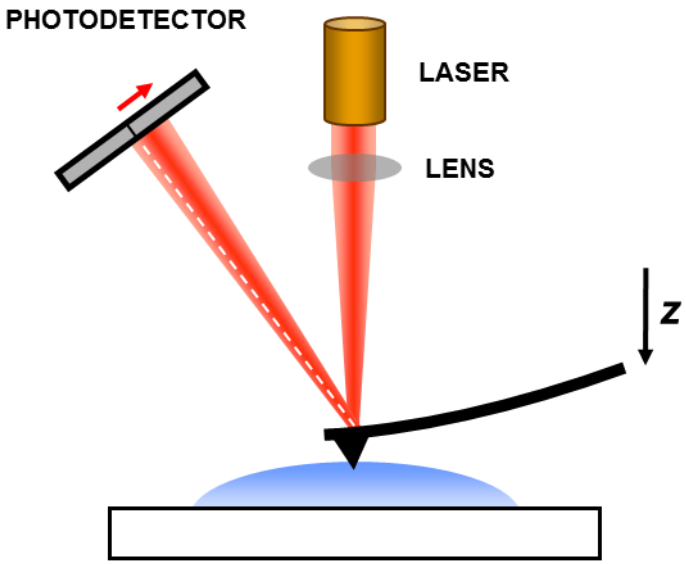
Engler *et al*. Cell, 2006

Effects of the Decellularization Method on the Local Stiffness of Acellular Lungs

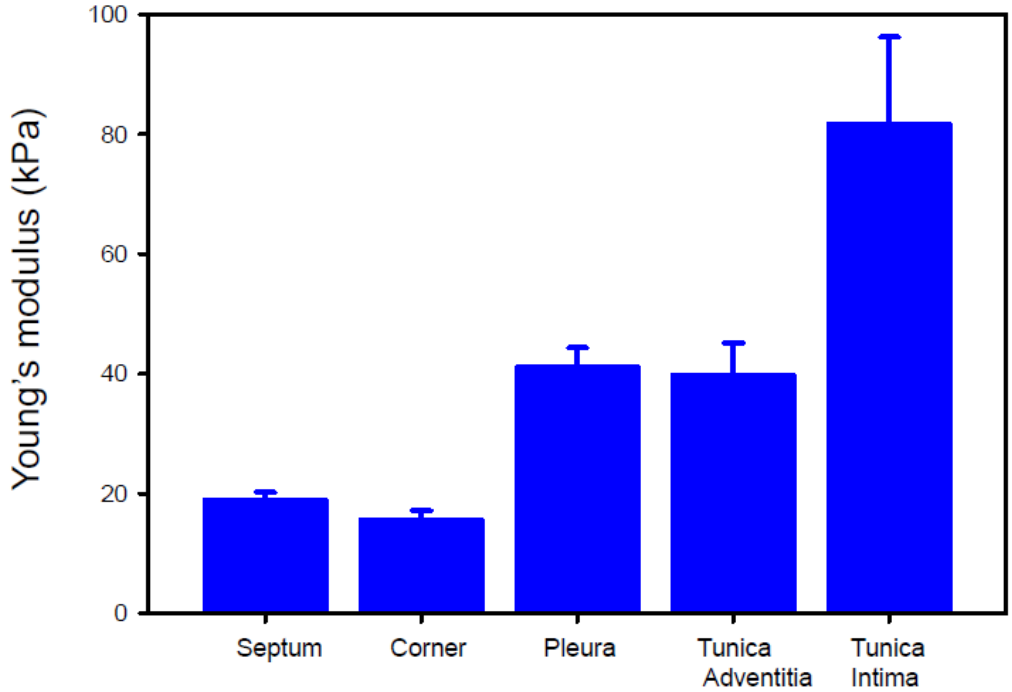
Esther Melo, BSc,[1–3] Elena Garreta, PhD,[1–3] Tomas Luque, BSc,[1,2,4] Joaquin Cortiella, PhD,[5] Joan Nichols, PhD,[5] Daniel Navajas, PhD,[1,2,4] and Ramon Farré, PhD[1–3]

TISSUE ENGINEERING: Part C
Volume 20, Number 5, 2014

Microscopía de Fuerza Atómica



Heterogeneidad de la rigidez de la matriz extracelular pulmonar



Physiological Reports

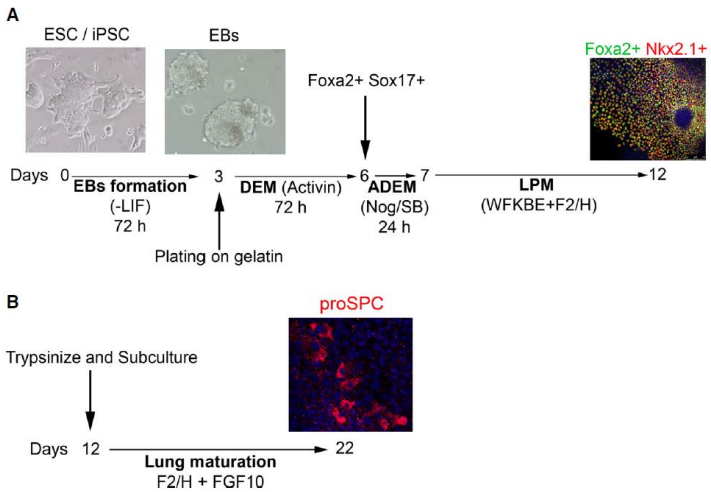
Open Access

Physiological Reports ISSN 2051-817X

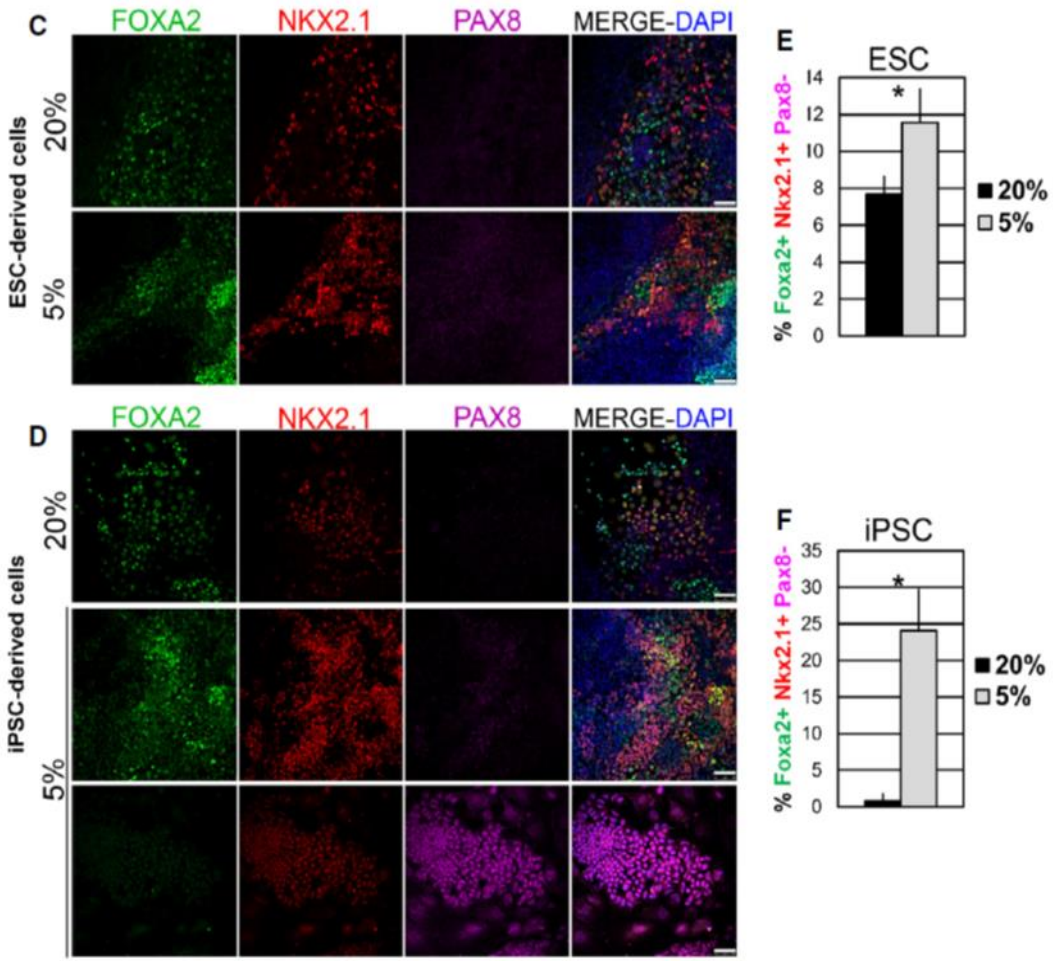
ORIGINAL RESEARCH

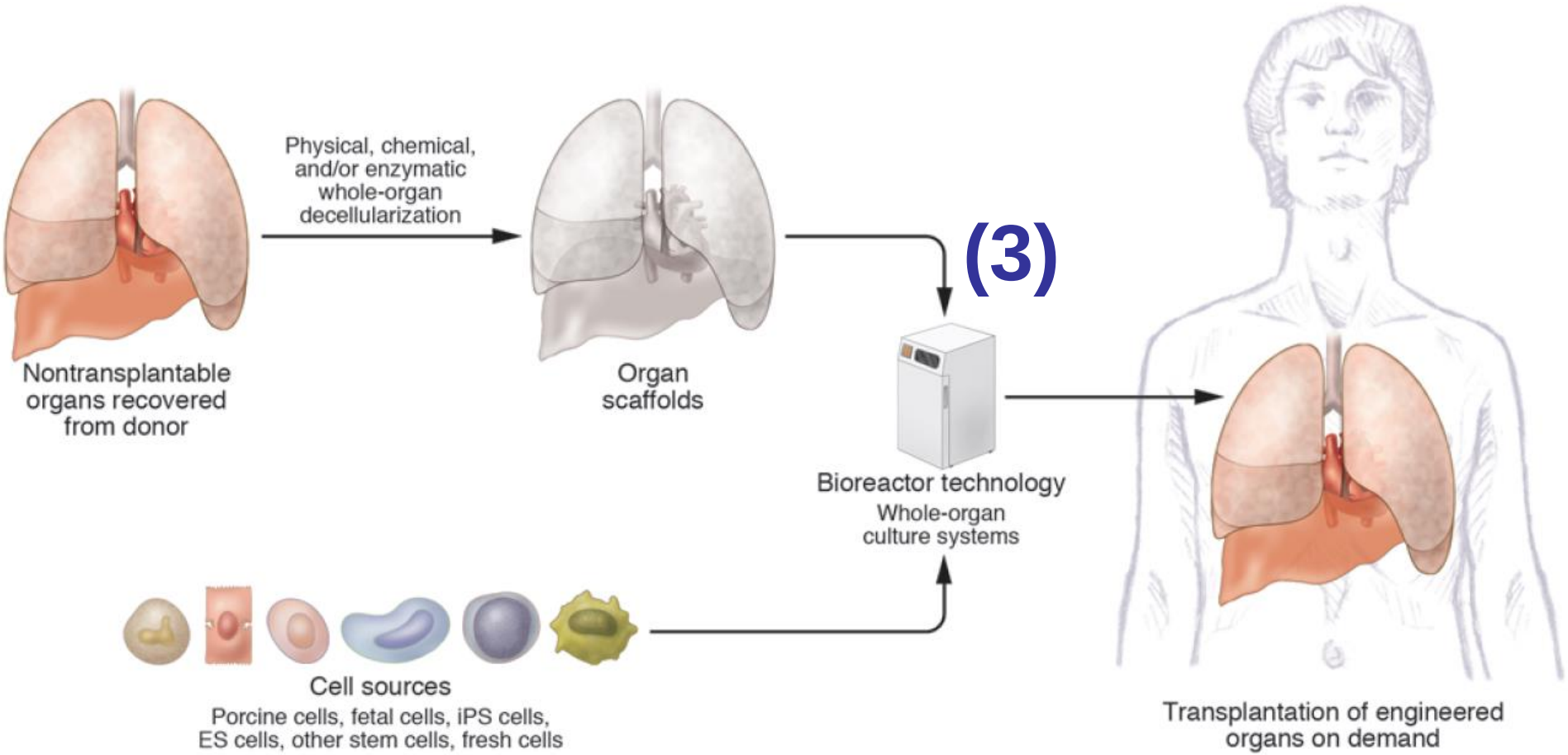
Low oxygen tension enhances the generation of lung progenitor cells from mouse embryonic and induced pluripotent stem cells

Elena Garreta[1,2,3], Esther Melo[1,2,3], Daniel Navajas[1,2,4] & Ramon Farré[1,2,3]

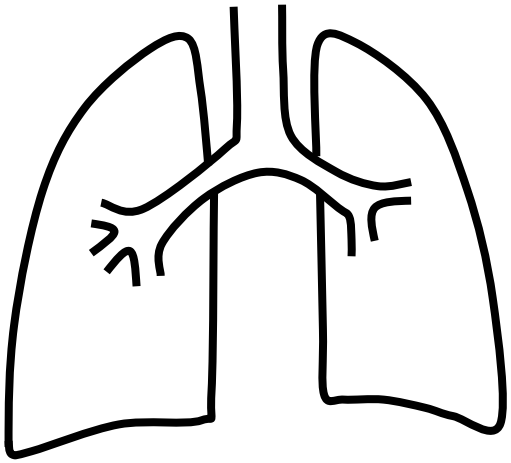


20% O_2 **5% O_2**





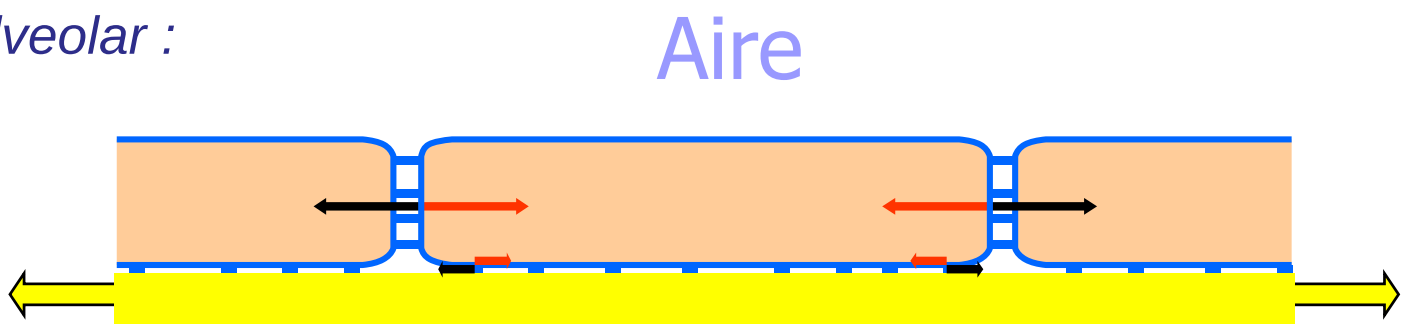
Soto-Gutierrez et al. J Clin Invest. 2012;122(11):3817–3823

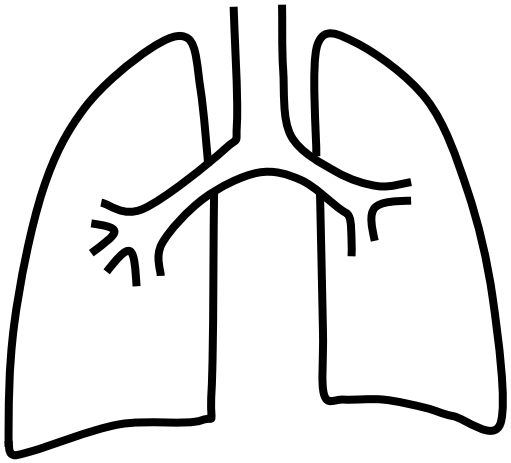


Estímulo mecánico:

- *Deformación cíclica*

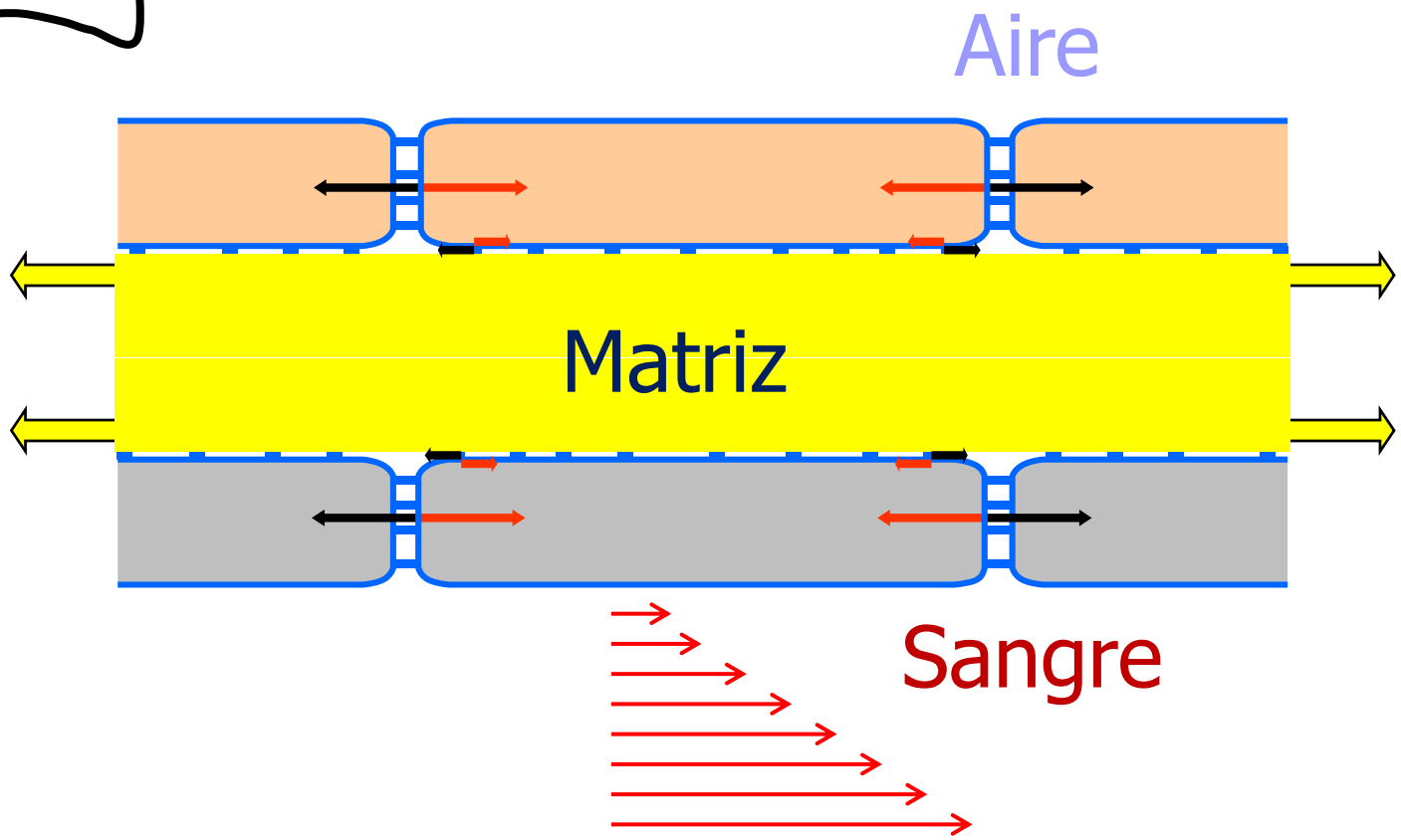
Epitelio alveolar :





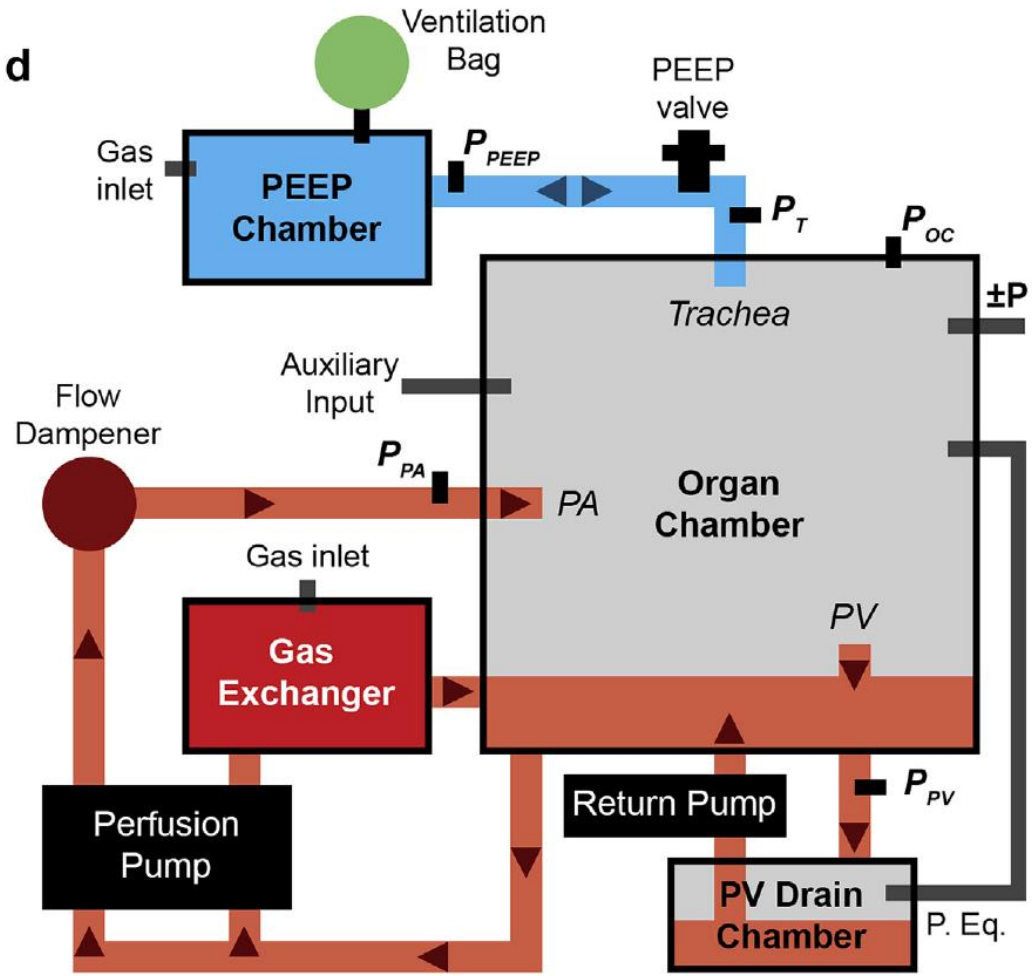
Estímulo mecánico:

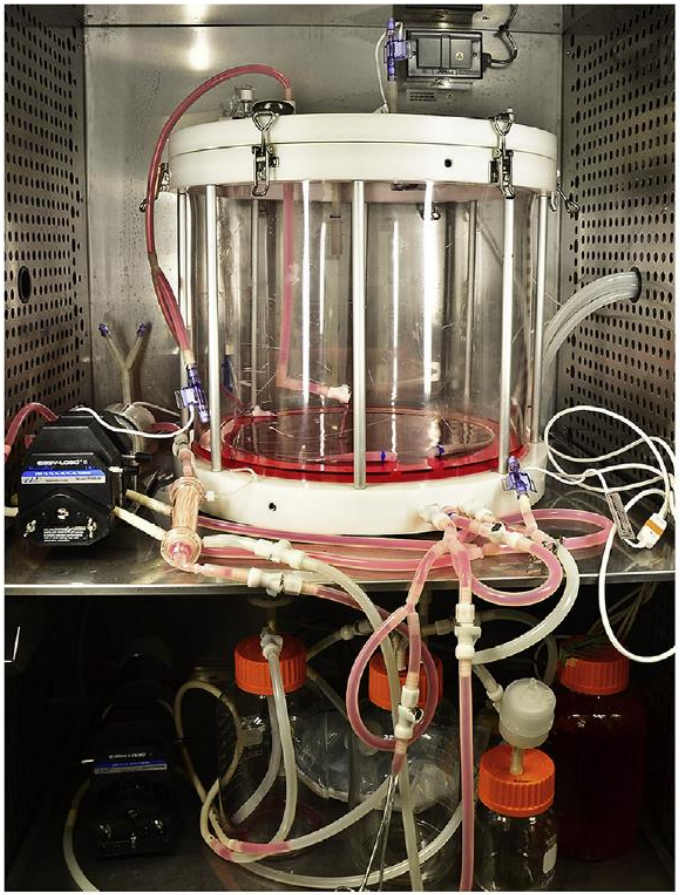
- *Deformación cíclica*
- *Esfuerzo de corte*



Bioreactors for whole lungs

Clinical-scale, long-term lung bioreactor





Charest et al. Biomaterials 52 (2015) 79e87

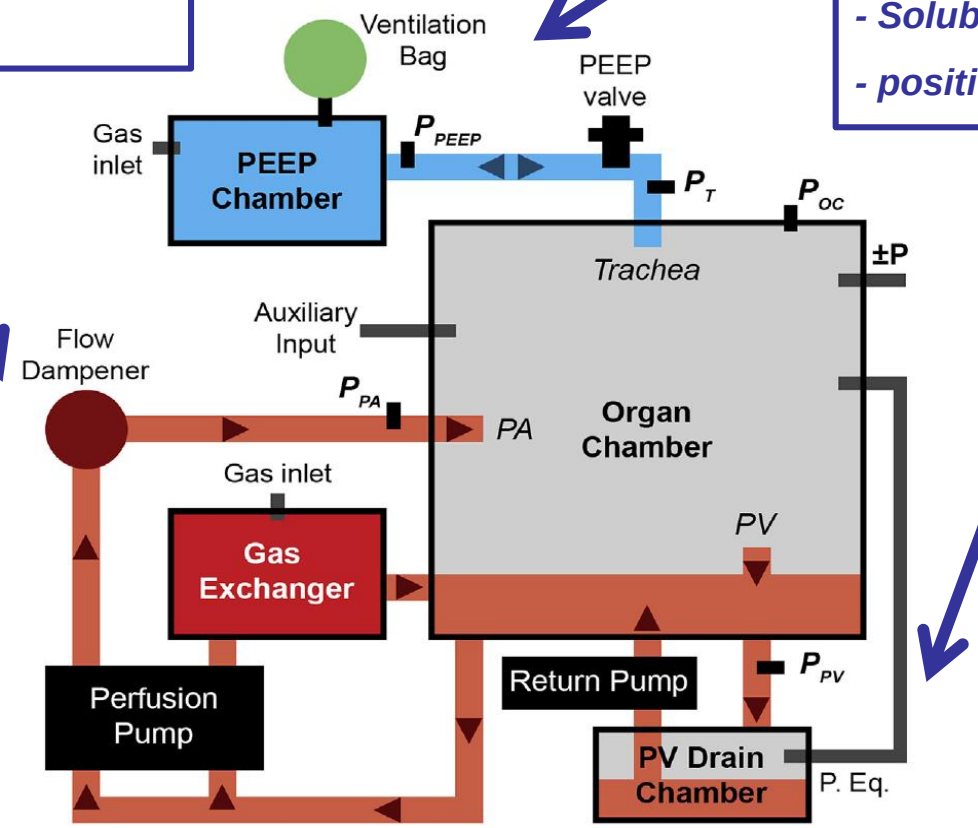
Bioreactors for whole lungs: optimization?

PERFUSION:

- continuos/pulsatile flow?
- arterial pulmonary pressure?
- perfusion flow?
- O_2, CO_2 ?
- Soluble factors?

VENTILATION:

- air/líquid ventilation?
- tidal volume, frequency?
- PEEP?
- volume, frequency?
- O_2, CO_2
- Soluble factors?
- positive/negative pressure?



Charest et al.
Biomaterials 52 (2015) 79e87

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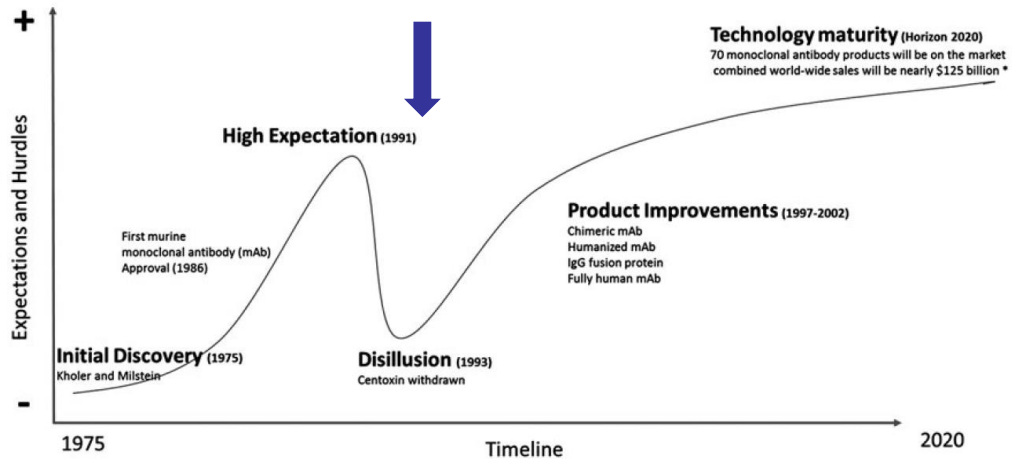
How difficult will be to succeed in lung bioengineering?

How many "death valleys"?

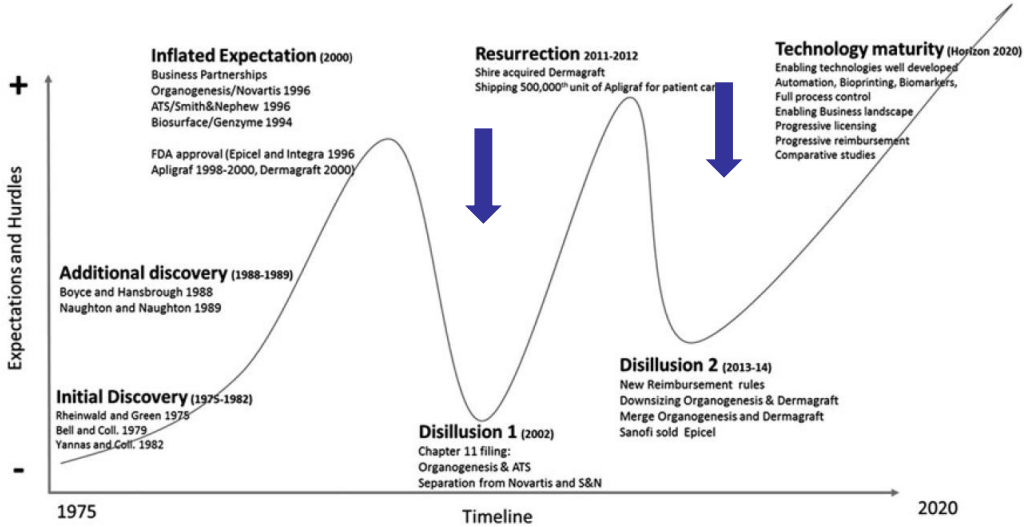
Typical "death valley"

(therapeutic monoclonal antibody)



Roadmap of bioengineered

skin products



Ronfard et al. Tissue Engineering B, 2016

CONCLUSIONS

Lung bioengineering has recently emerged as a potential alternative to increase the availability of viable lungs for transplantation.

A convenient approach for lung biofabrication seems to be the recellularization of acellular lung scaffolds with stem cells.

Although some experimental works have provided proof of concept, there are still an enormous number of questions to be posed and solved.

In particular, the best procedures for lung biofabrication in a bioreactor are still to be determined.

CONCLUSIONS

Given that lung bioengineering is a novel and challenging concept still in its scientific childhood, it will take a long way before its application in patients.

LUNG TRANSPLANTATION

1894, O. Lan: organ transplantation as a theoretical concept.

1940's, V. Petrovich: first lung transplantation in a dog.

1960's, J. Hardy: first lung transplantation in patients

1980's, J. Cooper: start clinical transplantation program

~~45 years~~

~~25 years~~

~~20 years~~

BIOENGINEERED LUNG TRANSPLANTATION: in 10 years?

AGRADECIMIENTOS

Jordi Otero

Daniel Navajas

Isaac Almendros

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