# Bioengineering in Organ Transplantation:

## *Focus on Liver*

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# Successful First Organ Transplantation History

**1905**: First successful cornea transplant by Eduard Zirm

**1954**: First successful kidney transplant by Joseph Murray (Boston, U.S.A.)

**1966**: First successful pancreas transplant by Richard Lillehei and William Kelly (Minnesota, U.S.A.)

**1967**: First successful liver transplant by Thomas Starzl (Denver, U.S.A.)

**1967**: First successful heart transplant by Christiaan Barnard (Cape Town, South Africa)

**1981**: First successful heart/lung transplant by Bruce Reitz (Stanford, U.S.A.)

**1983**: First successful lung lobe transplant by Joel Cooper (Toronto, Canada)

**1986**: First successful double-lung transplant (Ann Harrison) by Joel Cooper (Toronto, Canada)

**1995**: First successful laparoscopic live-donor nephrectomy by Lloyd Ratner and Louis Kavoussi (Baltimore, U.S.A.)

**1998**: First successful live-donor partial pancreas transplant by David Sutherland (Minnesota, U.S.A.)

**1998**: First successful hand transplant (France)

**2005**: First successful partial face transplant (France)

**2006**: First jaw transplant to combine donor jaw with bone marrow from the patient, by Eric M. Genden (Mount Sinai Hospital, New York)

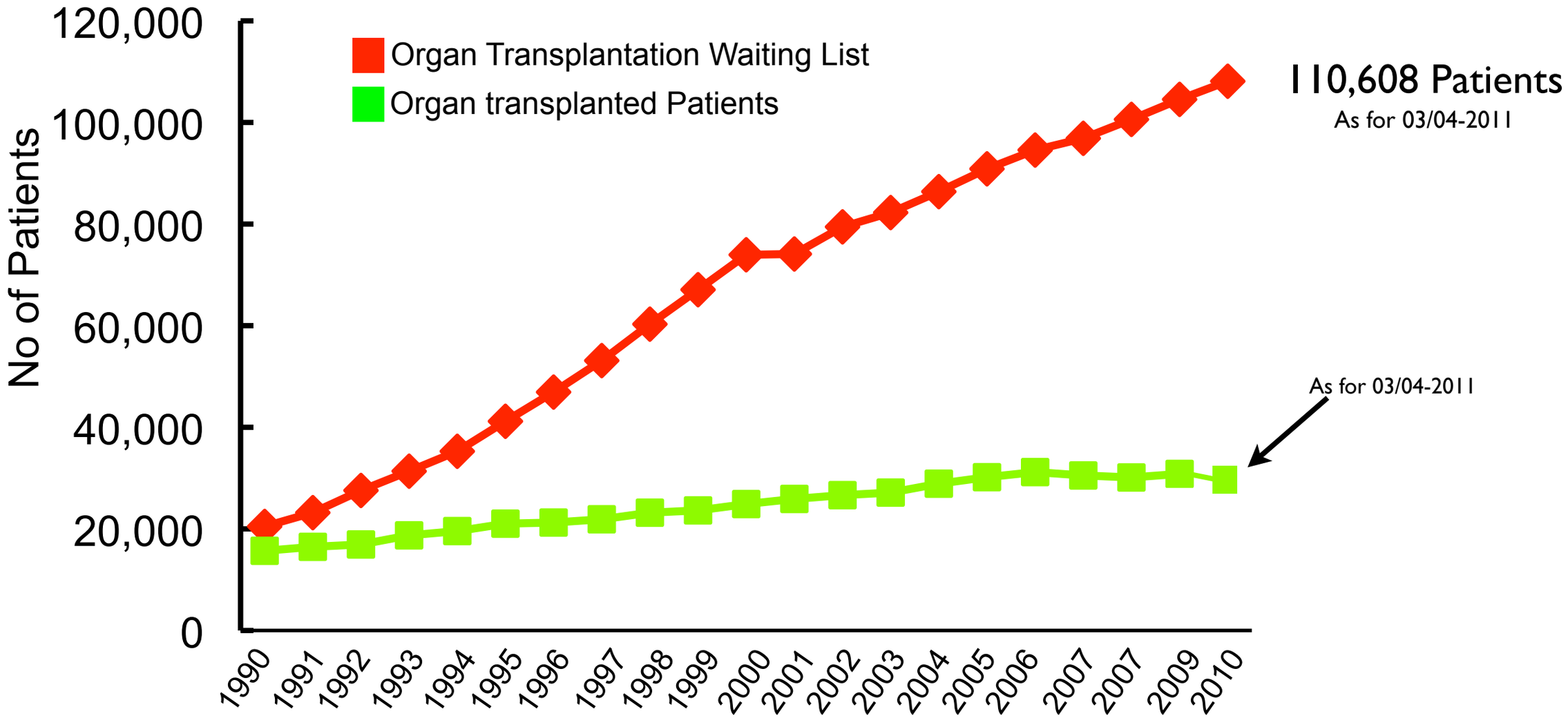
**2008**: First successful complete full double arm transplant by Edgar Biemer, Christoph Höhnke and Manfred Stangl (Technical University of Munich, Germany)

**2008**: First transplant of a human windpipe using a patient's own stem cells, by Paolo Macchiarini (Barcelona, Spain)

**2010**: First successful full face transplant by Dr Joan Pere Barret and team (Vall d'Hebron University Hospital of Barcelona, Spain)

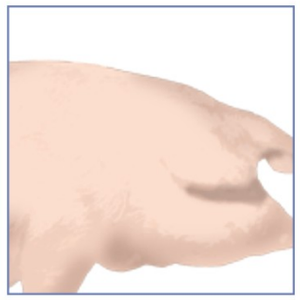
# Success!!

# Organ Transplantation Waiting List
# vs
# Transplanted Patients




United Network for Organ Sharing (http://www.unos.org/)

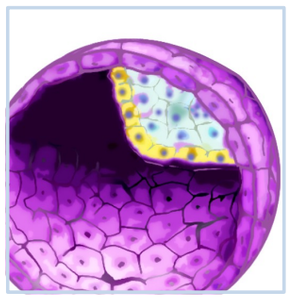
# Alternative Technologies to Organ Transplantation



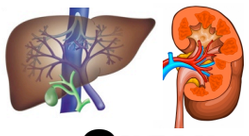
Pig Cells

Fetal Cells

iPS Cells



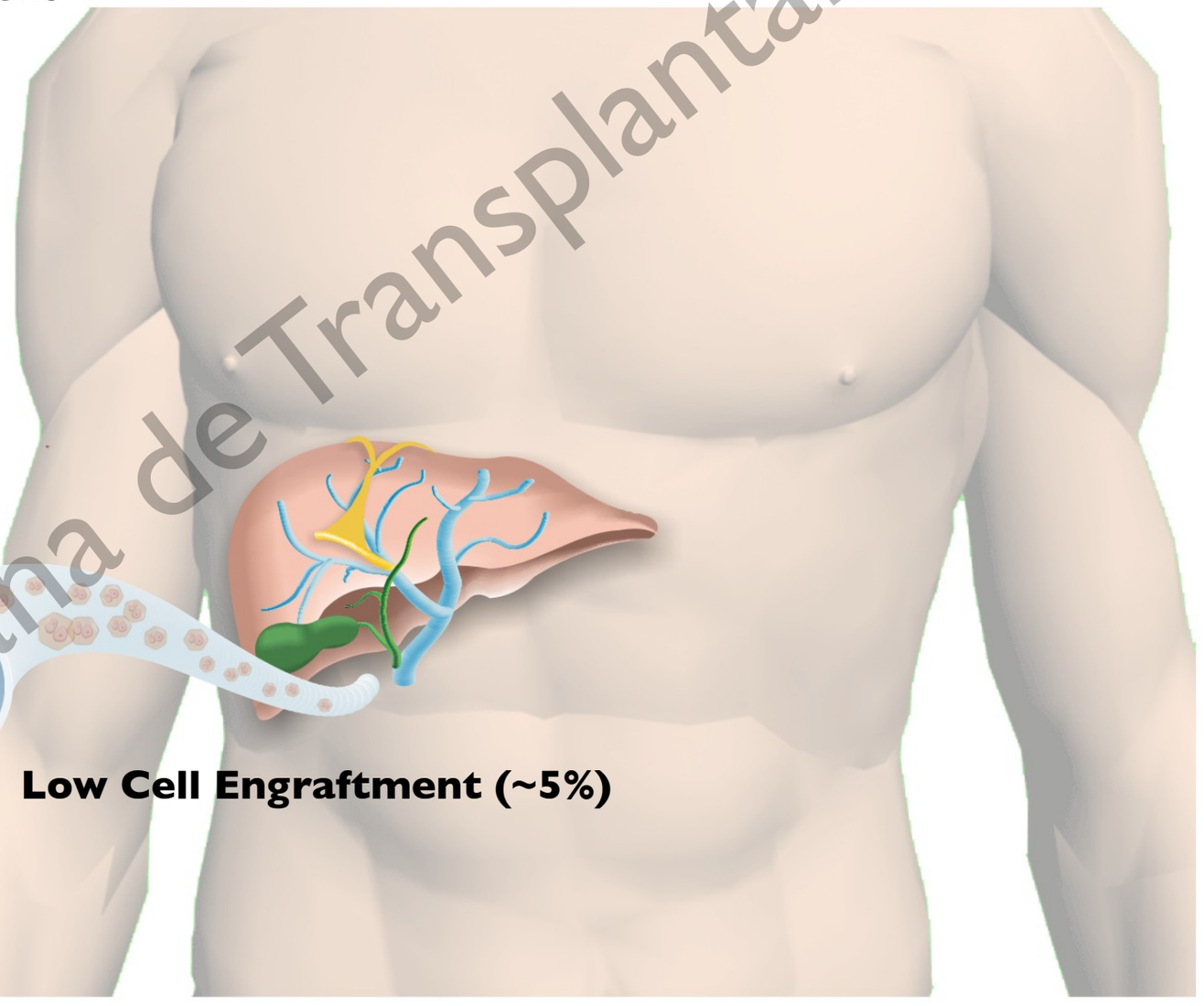
ES Cells



**Organ Xenotransplantation**

**Bio-Engineered Systems for Cell Delivery**

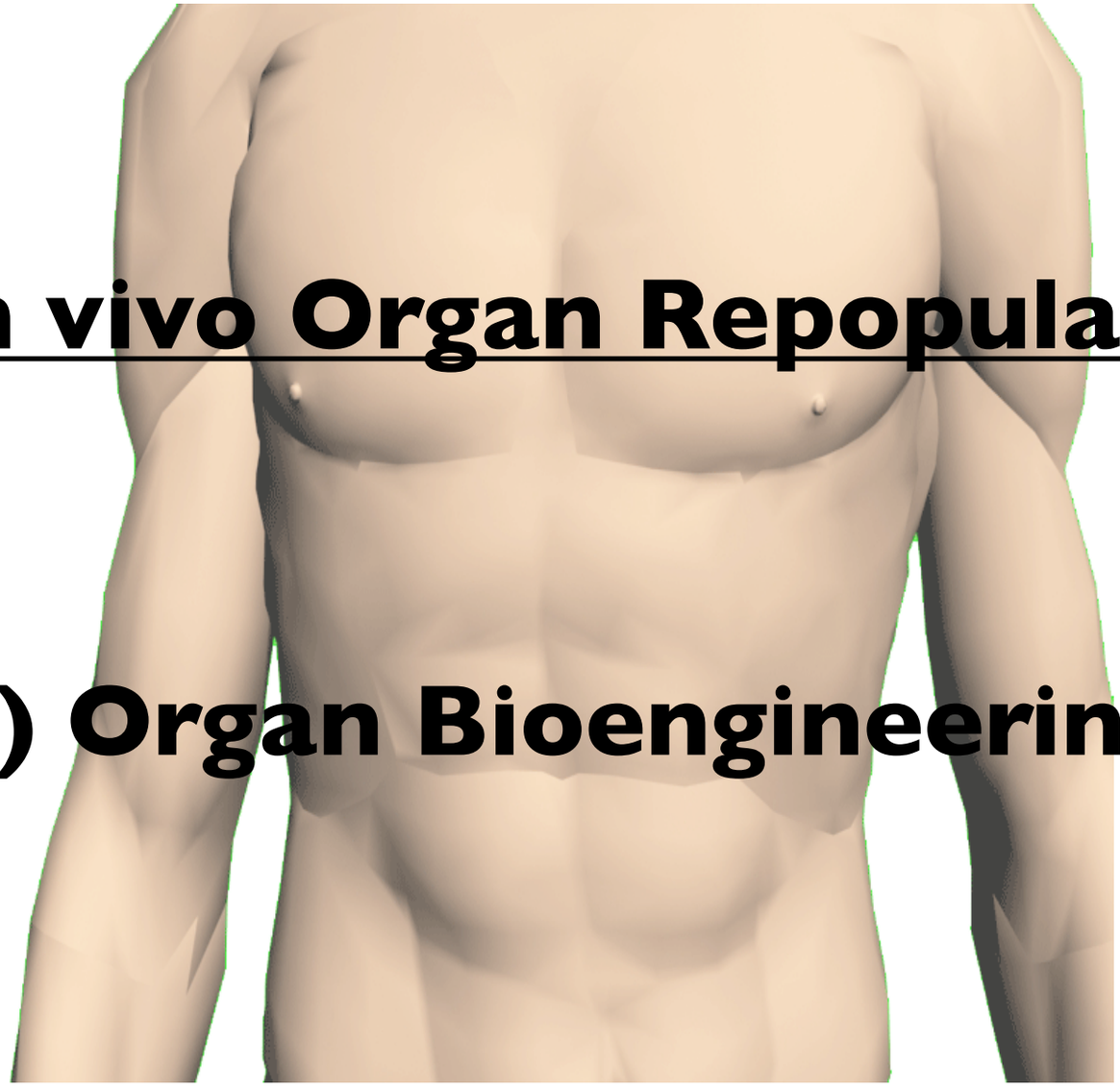
**Direct Cell Transplantation**



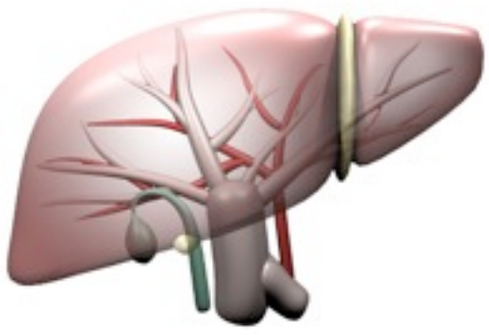



Soto-Gutiérrez A, Kobayashi N, et al. Nat Biotechnol. 2006;24(11):1412-9.
Soto-Gutierrez A, Navarro-Alvarez N, et al. Transplantation. 2007 Jan, 27;83(2):129-37.
Navarro-Alvarez N, Soto-Gutierrez A, et al. J Hepatol. 2010 Feb; 52(2):211-9.

# Regenerative Medicine Approaches for Organ Replacement



## a) In vivo Organ Repopulation



## b) Organ Bioengineering

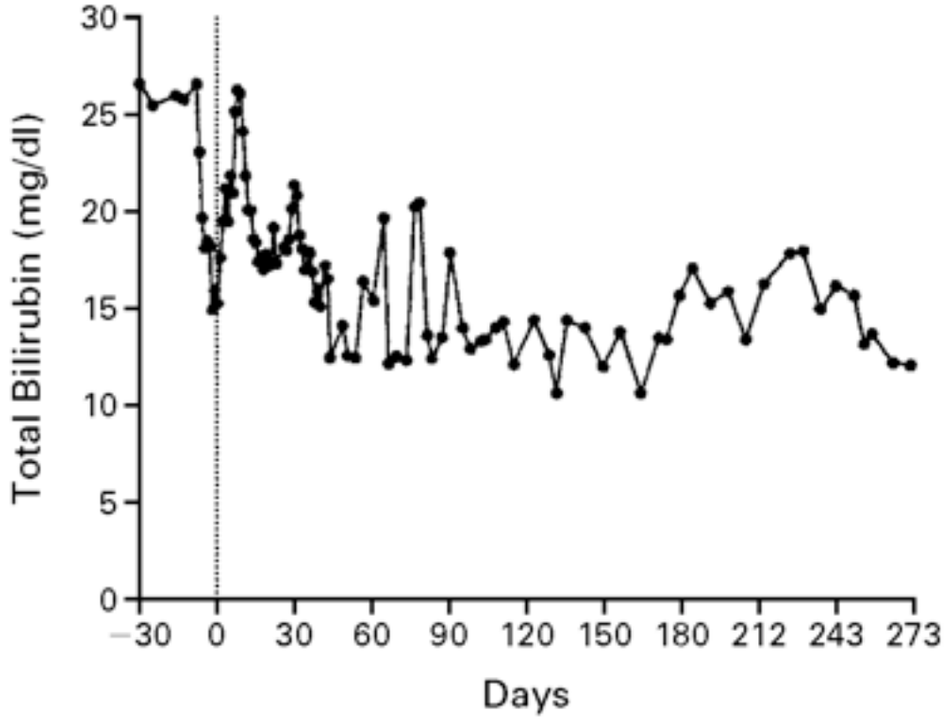
ORIGINAL ARTICLE

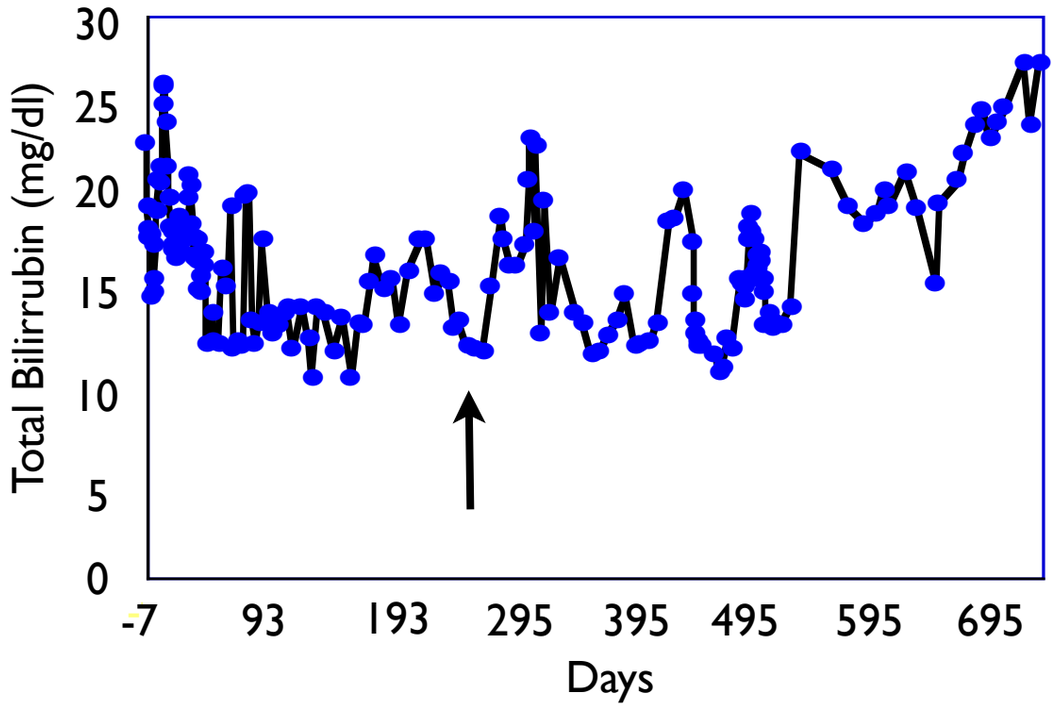
Brief Report

# Treatment of the Crigler–Najjar Syndrome Type I with Hepatocyte Transplantation

Ira J. Fox, M.D., Jayanta Roy Chowdhury, M.D., Stuart S. Kaufman, M.D., Timothy C. Goertzen, M.D., Namita Roy Chowdhury, Ph.D., Phyllis I. Warkentin, M.D., Kenneth Dorko, B.S., Bernhard V. Sauter, M.D., and Stephen C. Strom, Ph.D.

N Engl J Med 1998; 338:1422-1427 | May 14, 1998

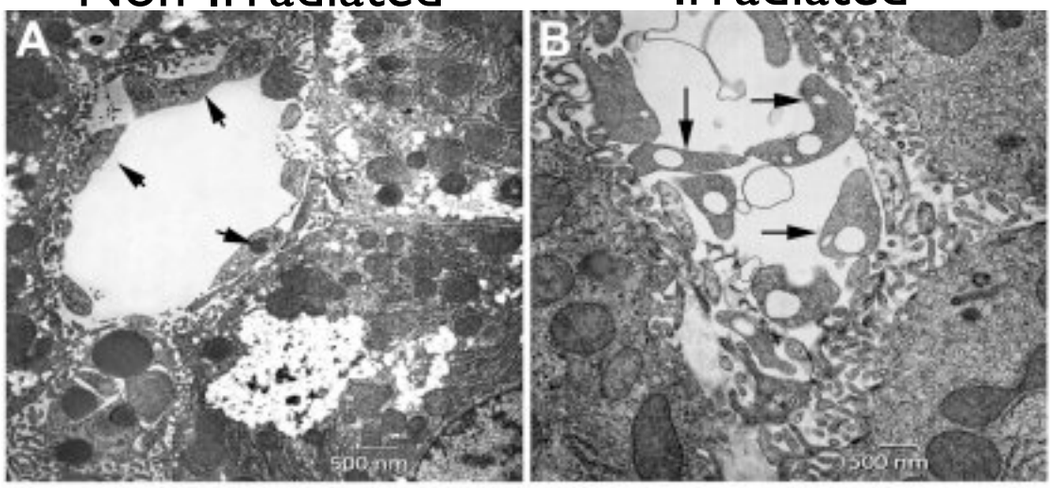



$7.5 \times 10^9$ hepatocytes were infused through the portal-vein catheter over a period of 15 hours.

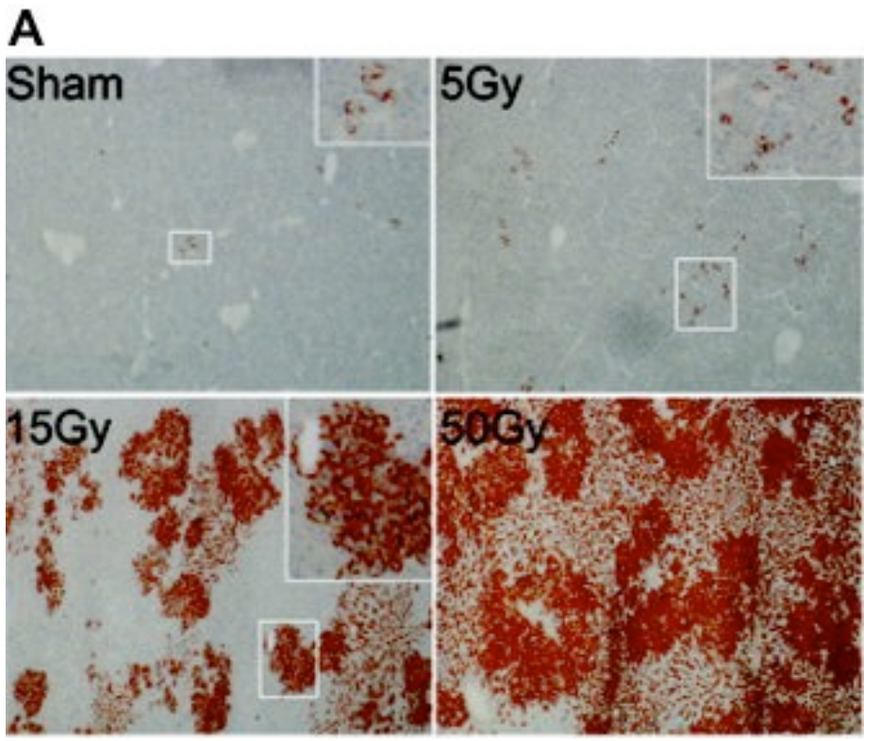
# Hepatic Irradiation Augments Engraftment of Donor Cells Following Hepatocyte Transplantation

Non-Irradiated | Irradiated




Disruption of Lining Endothelial Cells

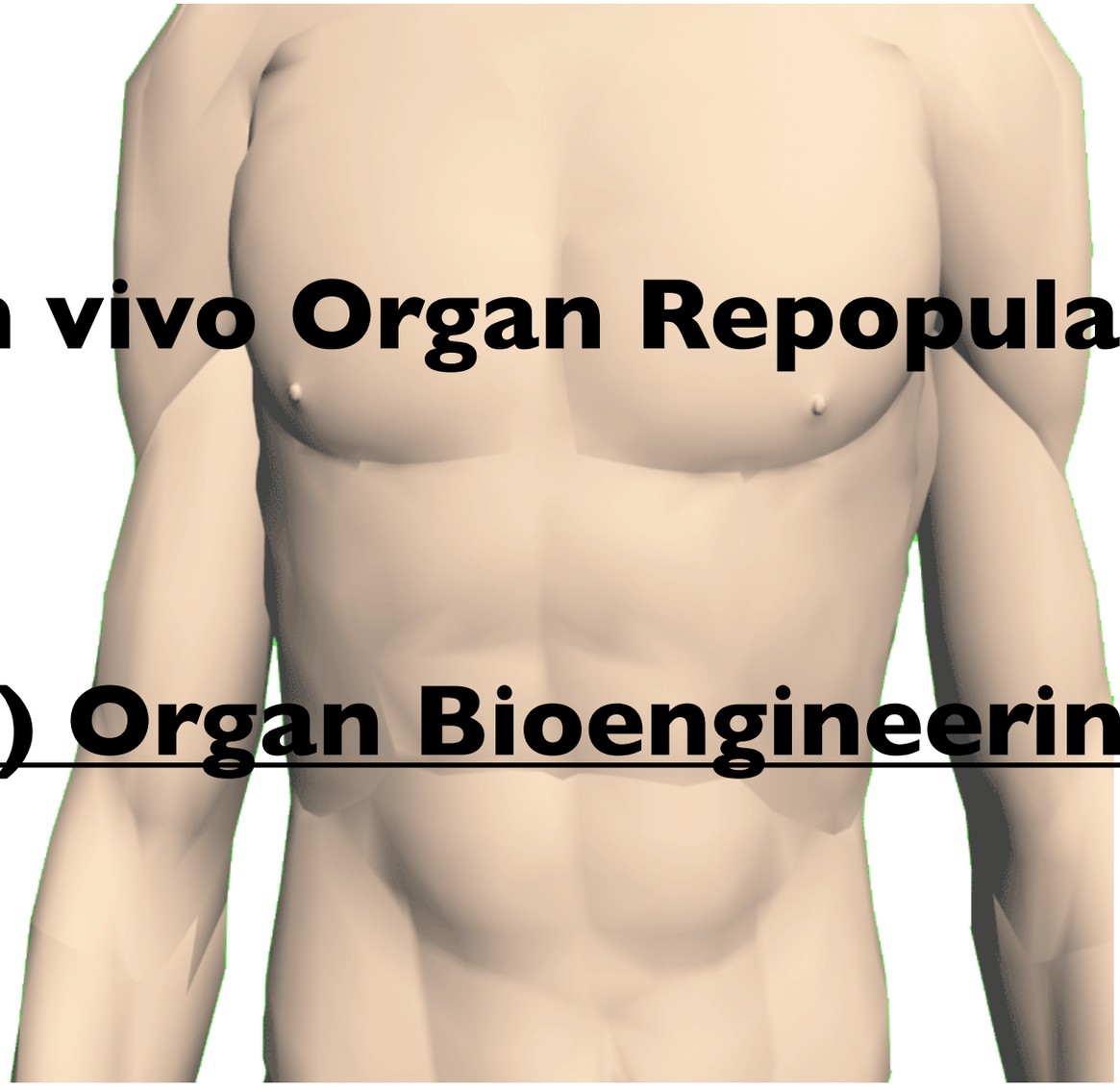
*Space!!!
*Growth Advantage!!!




Growth Advantage for Donor cells in the Irradiated Area

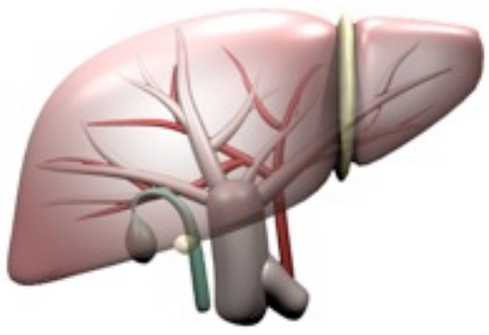
Fox IJ, Roy-Chowdhury J, Guha C. Hepatology. 2009 Jan;49(1):258-67

# Regenerative Medicine Approaches for Organ Replacement



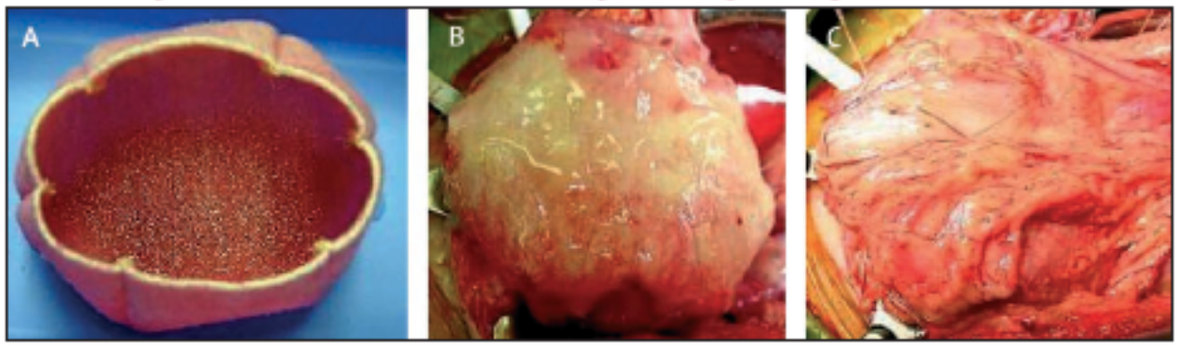
## a) In vivo Organ Repopulation

## b) Organ Bioengineering



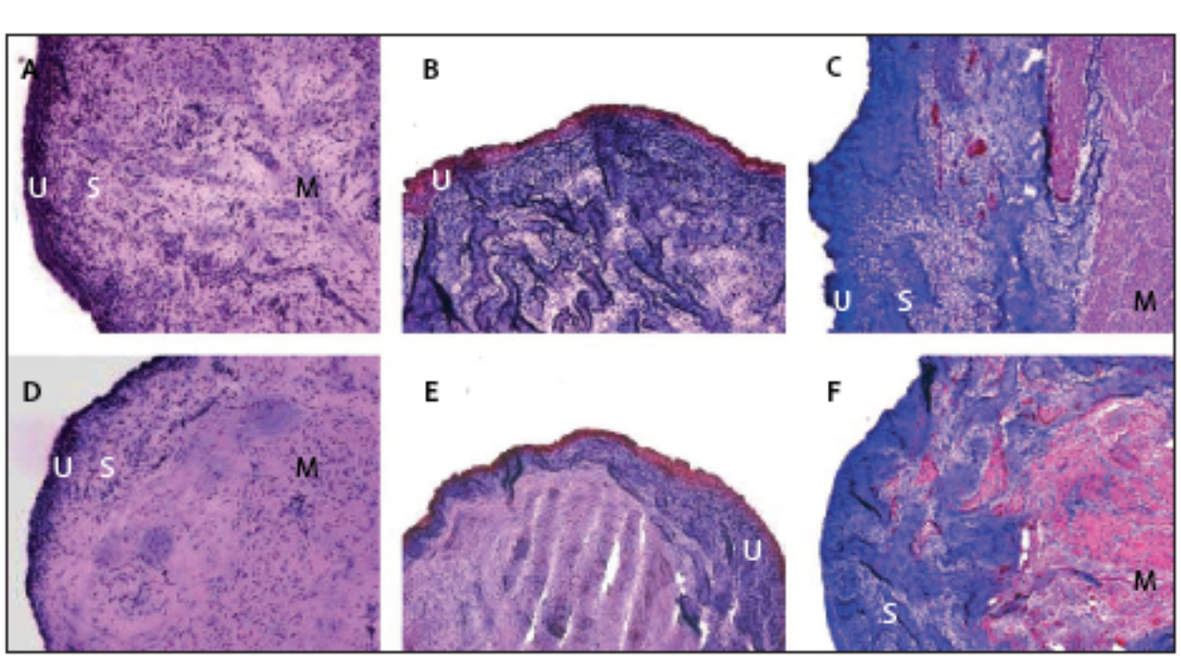
# Tissue-engineered autologous bladders for patients needing cystoplasty

*Anthony Atala, Stuart B Bauer, Shay Soker, James J Yoo, Alan B Retik*




Urothelial and muscle cells were grown in culture, and seeded on a biodegradable bladder-shaped scaffold made of collagen, or a composite of collagen and polyglycolic acid.

Seven patients with myelomeningocele, aged 4-19 years, with high-pressure or poorly compliant bladders were transplanted with Engineered Bladders!




Engineered Bladder

Native Bladder

Cytoscopic Biopsies of implanted engineered bladders

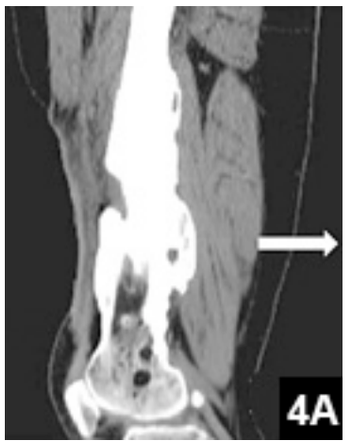
Atala A. Lancet. 2006 Apr 15;367(9518):1241-6.

# Clinical Application of an Acellular Biologic Scaffold for Surgical Repair of a Large, Traumatic Quadriceps Femoris Muscle Defect

Traumatic Injury with Muscle loss

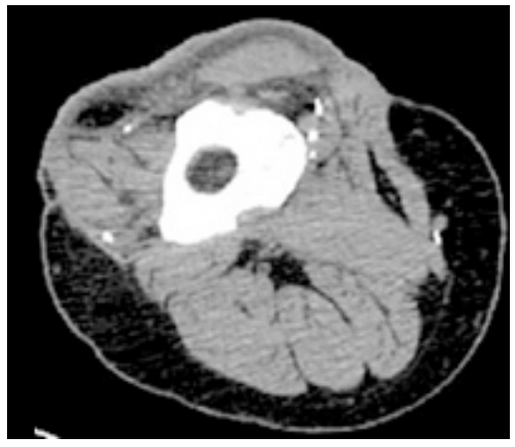



Muscle Reconstruction and Regeneration by surgical implantation of a multi-layered scaffold composed of extracellular matrix derived from porcine intestinal submucosa.

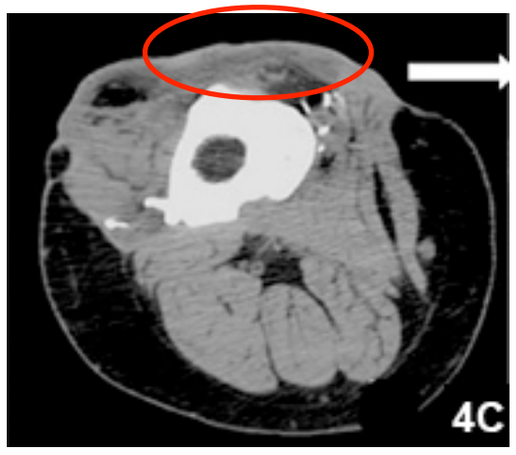



5m Before Bio-engineered Scaffold Implantation


9m After Bio-engineered Scaffold Implantation



5m Before Bio-engineered Scaffold Implantation



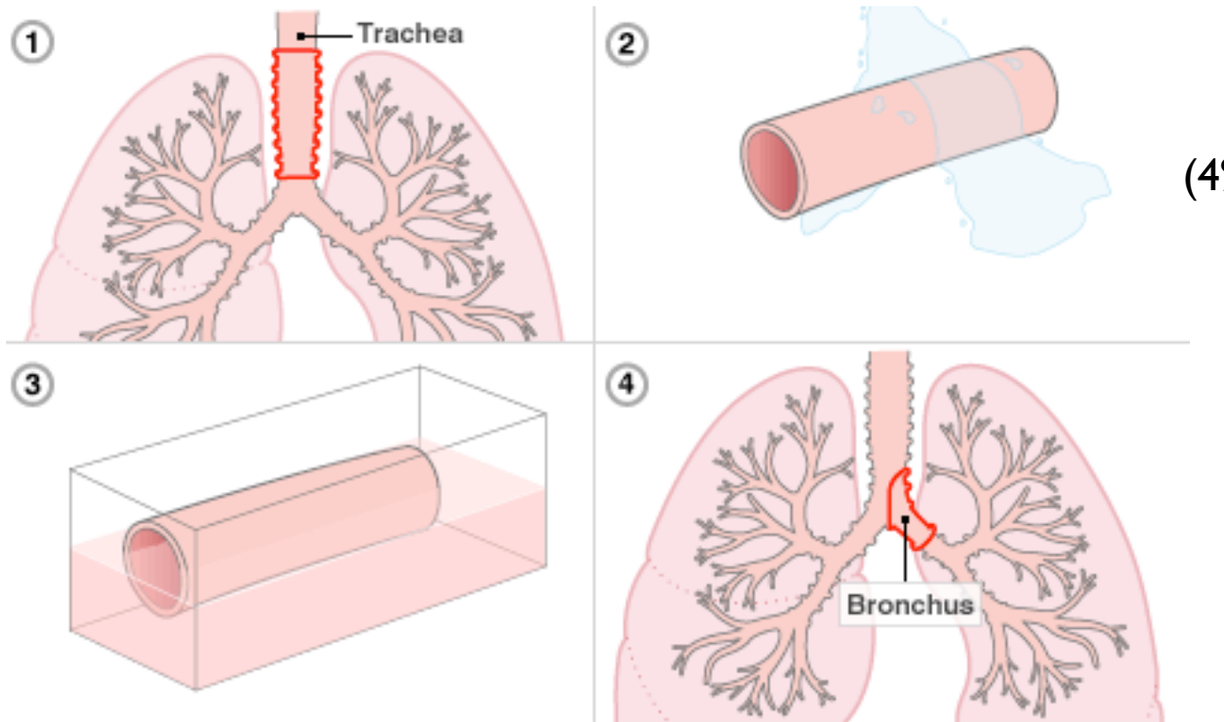

9m After Bio-engineered Scaffold Implantation

Mase VJ, Badylak S, Walters TJ. Orthopedics. 2010

# 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

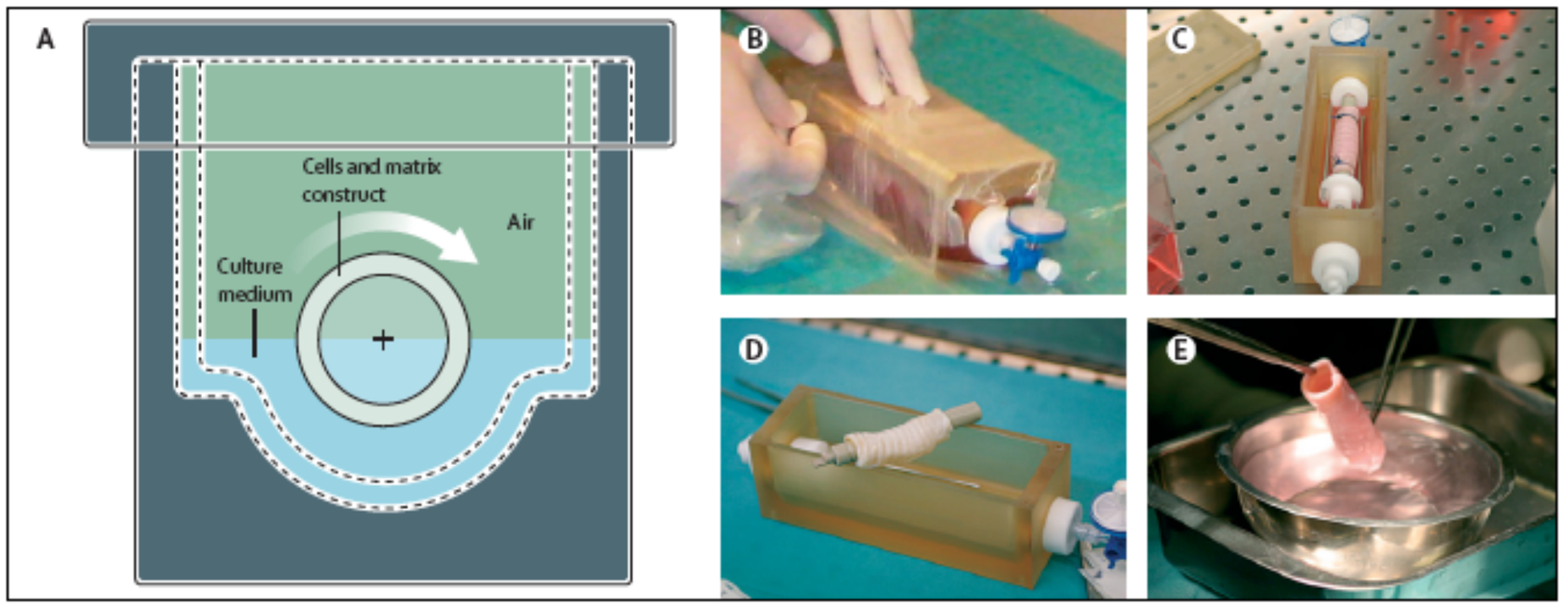
Extraction of Donor trachea



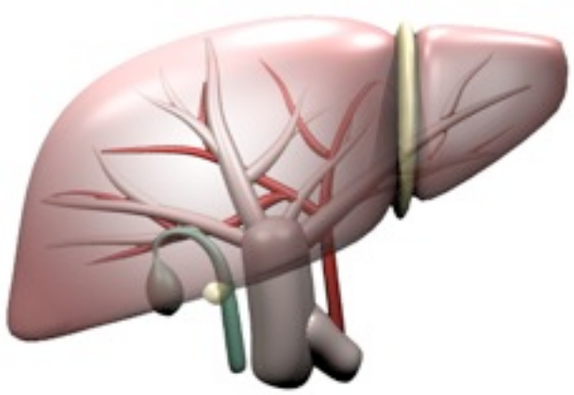

Decellularization of the Tissue
(4% sodium deoxycholate&DNase-I)

Recellularization with recipient's epithelial cells and chondrocytes

Transplantation of Re-engineered Traquea




Clinical transplantation of a tissue-engineered airway. Macchiarini P, et al. Lancet. 2008 Dec 13;372(9655):2023-30.



# Solid Organ Decellularization

# Mode of Actions of Different Decellularization Methods

## Physical

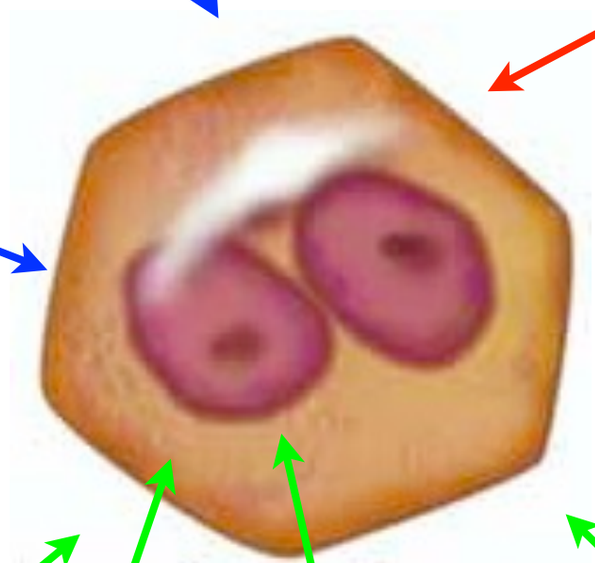
ECM can be easily disrupted!!!

**Mechanical Force**
**Mechanical Agitation**

Pressure, sonication can cause cell lysis

**Snap Freezing**

Intracellular ice crystals disrupt cell membrane



## Enzymatic

Prolonged exposure can disrupt ECM (laminin, elastin, fibronectin)!!!

**Trypsin**

Cleaves peptide bond Arg and Lys

**Endonucleases**

Catalyze the hydrolisis of the interior bonds of ribo- and desoxyribonucleotide chains

**Exonucleases**

Catalyze the hydrolisis of the terminal bonds of ribo- and desoxyribonucleotide chains

**EDTA, EGTA**

Disruption of cell adhesion to ECM

**Hypotonic and Hypertonic sol.**

Cell lysis by osmotic shock

**Tri(n-butyl)phosphate**

Disrupts protein-protein interactions

**Alkaline, Acid sol.**

Disrupts nucleic acids

**Triton X-100**

Disrupts lipid-lipid, lipid-protein interactions

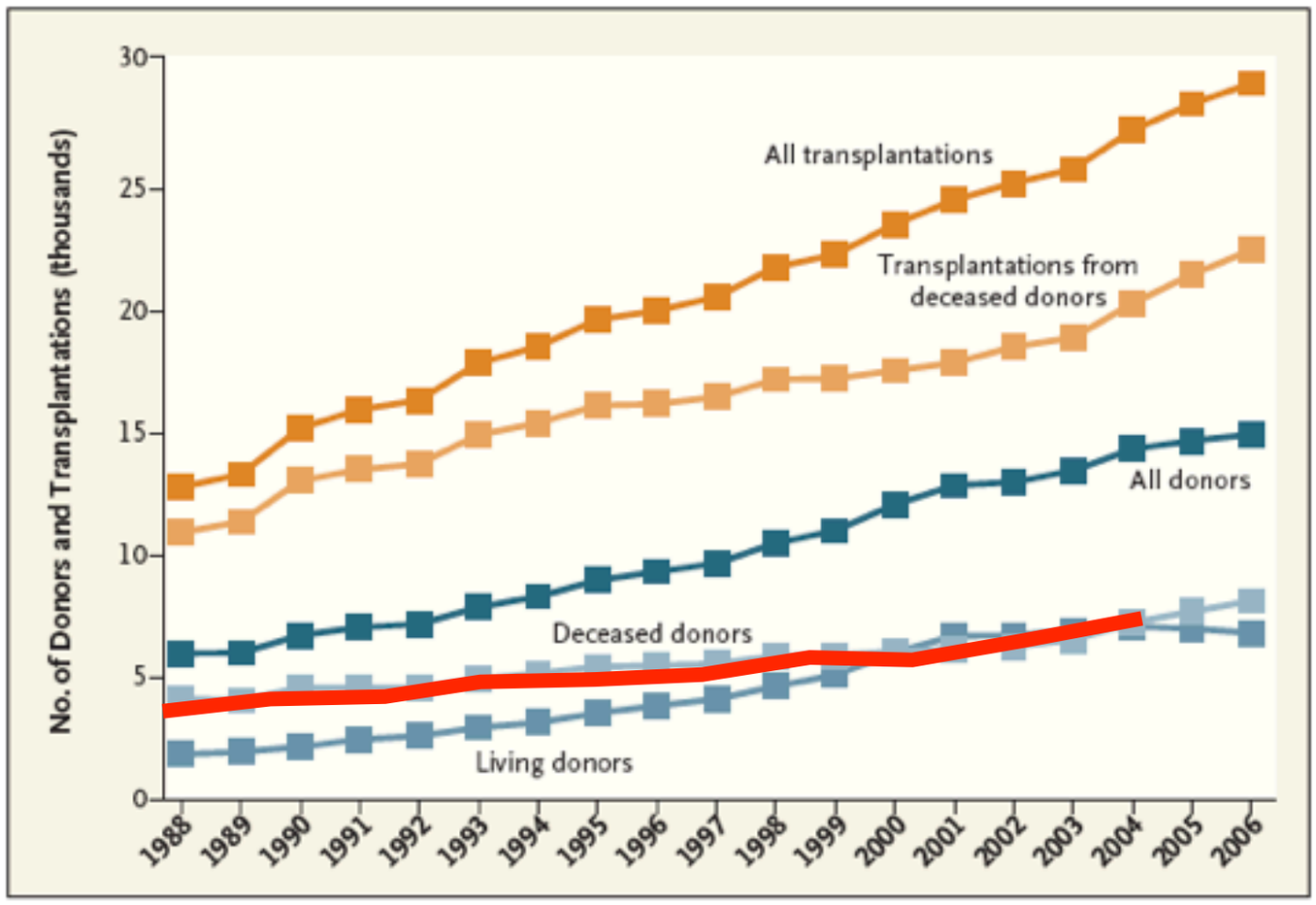
**SDS, Triton X-200**

Denature Proteins

**CHAPS, SB-10, SB-16**

## Chemical

# Organs After Cardiac Death




**Organ Donors and Transplantations in the United States, 1988–2006.**
Data are from the Organ Procurement and Transplantation Network.
Steinbrook R, 2007. NEJM. 357;3

In US, about 40% of the eligible deaths never became actual donors and those organs are currently discarded; the estimated number of such potential donors is 250,000 per year.

Even if some of these organs are reconditioned for transplantation, the remaining organs would provide ample supply for organ engineering technologies.


Punch, J.D., et al., Organ donation and utilization in the United States, 1996-2005. Am J Transplant,2007. 7(5 Pt 2): p. 1327-38.
Safar, P. Clinical death symposium. Crit Care Med, 1988. 16: p. 919-920.

# Protocols for Solid Organ Decellularization

**nature medicine** — Heart, 2008

10uM adenosine, 15 min → 1% SDS, 12 h → 1% Triton-X100, 30 min → PBS with antibiotics, 124 h

**nature medicine** — Liver, 2010

freeze -80°C → 4°C PBS, Over night → 0.01% SDS, 24 h → 0.1% SDS, 24 h → 1% SDS, 24 h → 1% Triton X-100, 30 min → 0.1% peracetic acid, 3h

**Science** — Lung, 2010

8mM CHAPS, 1M NaCl, 25mM EDTA, 2h → PBS with antibiotics → 90U/ml benzonase → PBS with 10% FBS

**nature medicine** — Lung, 2010

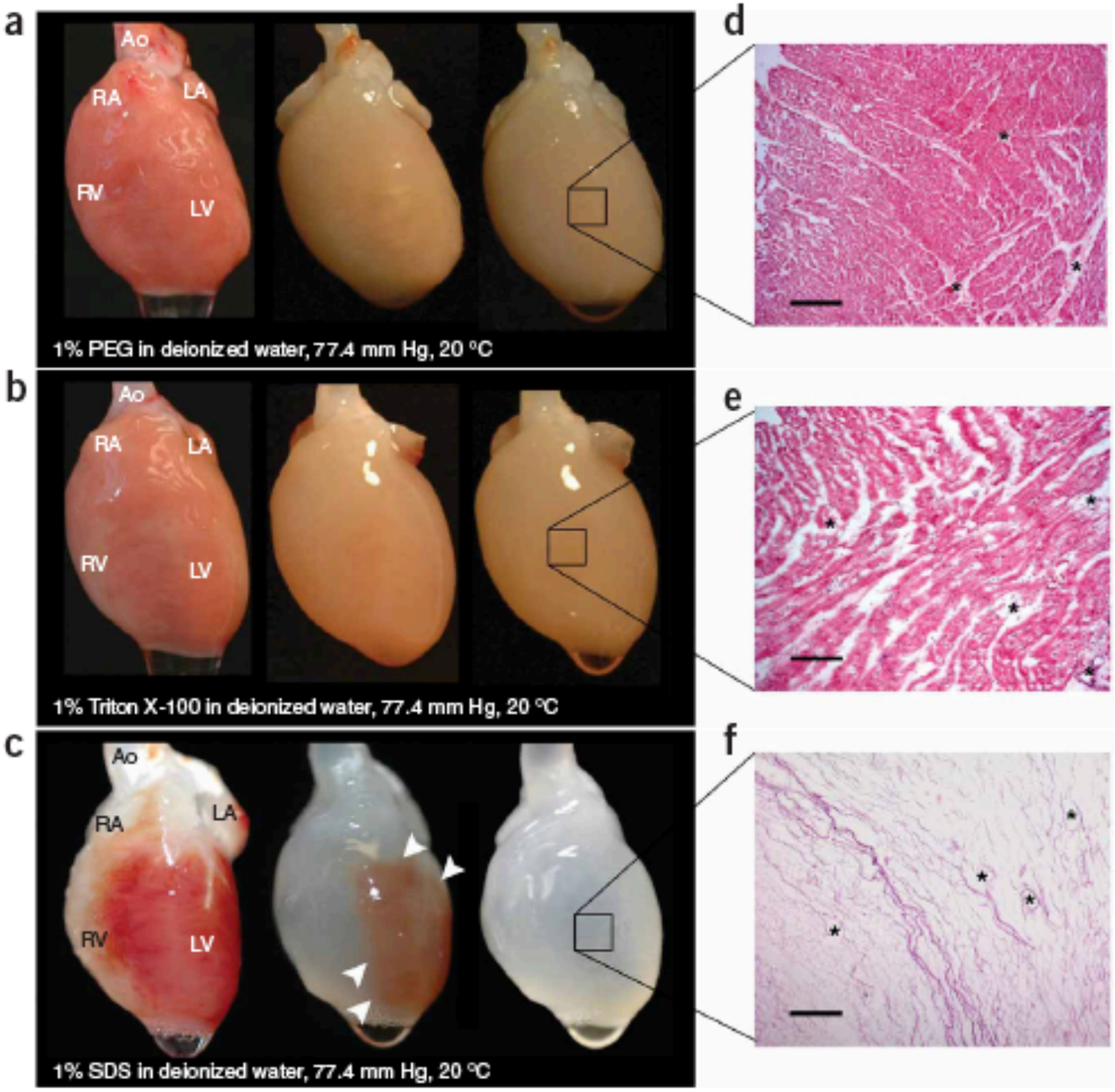
0.1% SDS, 120 min → 1% Triton X-100, 10 min → PBS with antibiotics, 72 h

**Tissue Engineering Part A** — Liver, 2011

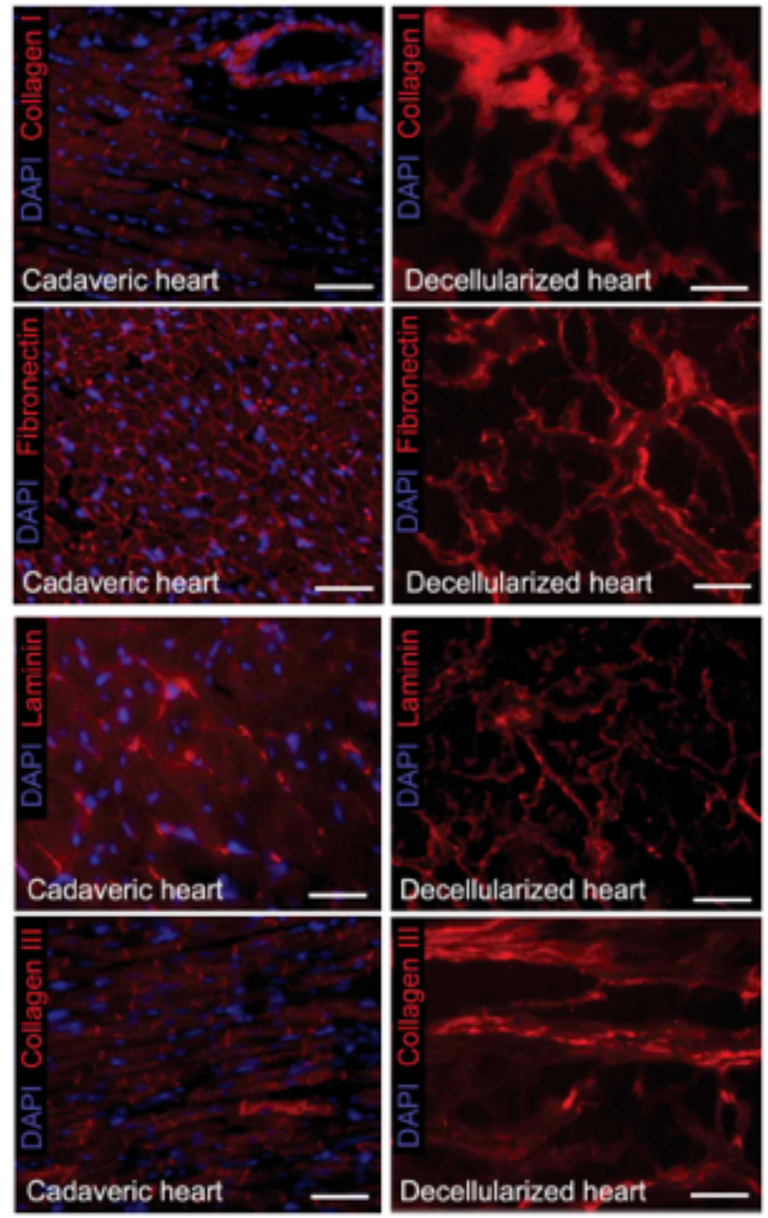
freeze -80°C, 24 h → 0.02% Trypsin, 2 h → 3% Triton X, 18-24 h → PAA, 1 h

nature medicine

# Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart



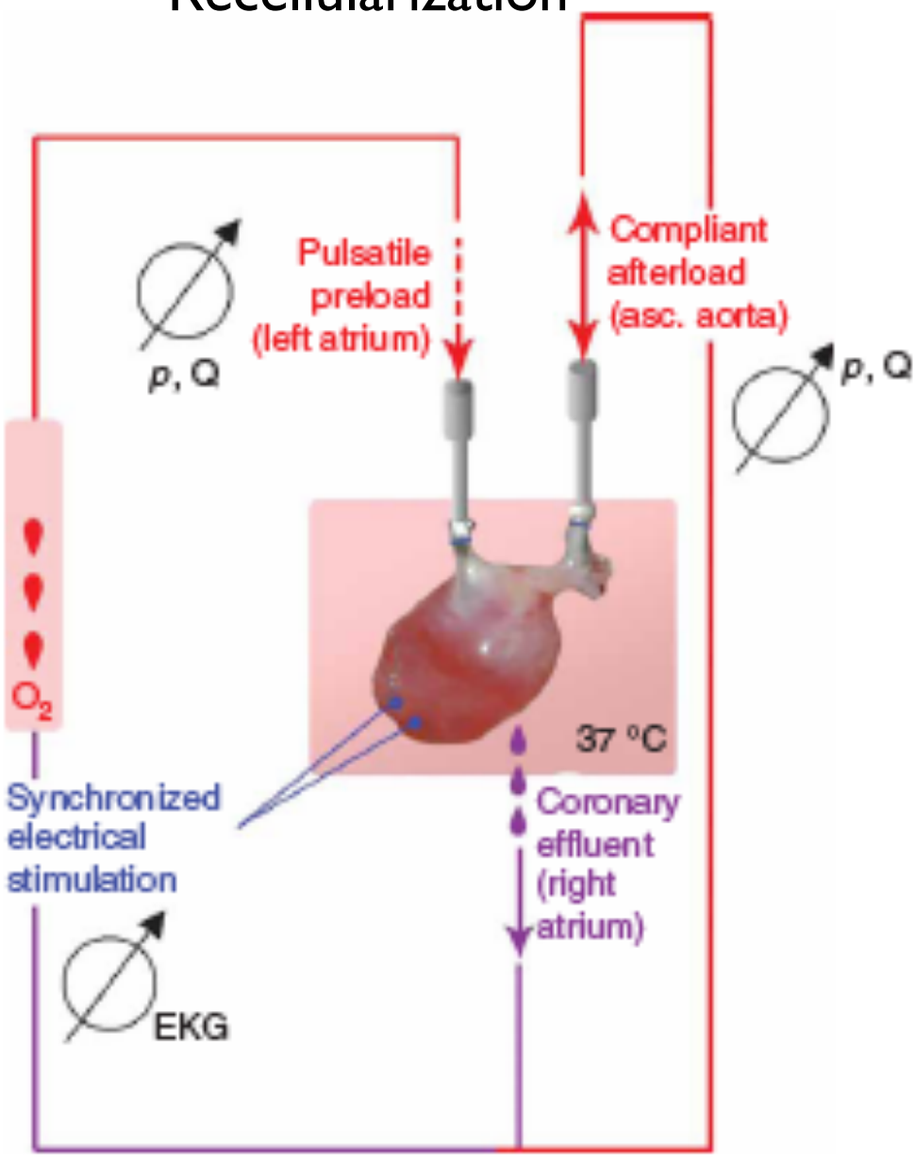

Characterization of Decellularized Heart by SDS



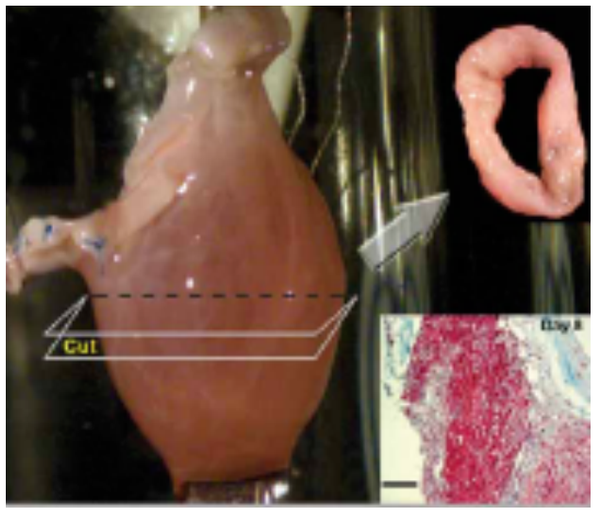

Ott HC, Taylor DA. Nat Med. 2008 Feb;14(2):213-21.

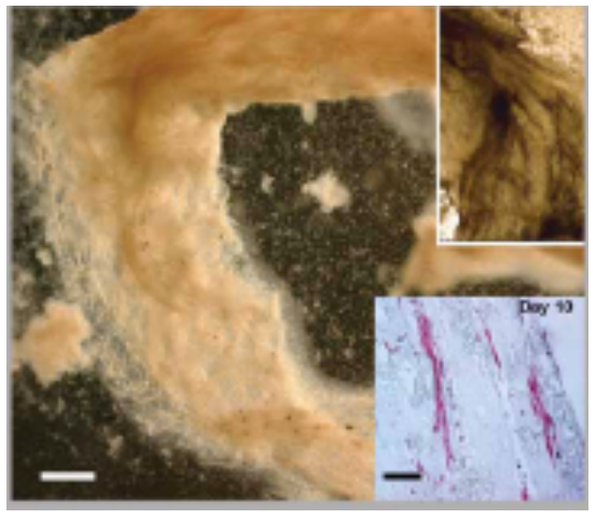
# Recellularization of Decellularized Heart using Neonatal Cardiac Cells

Bioreactor Model used for Recellularization




Perfusion System and Static Ring tissue culture System




2% Contraction Fraction Compare to a Normal Heart

Ott HC, Taylor DA. Nat Med. 2008 Feb;14(2):213-21.

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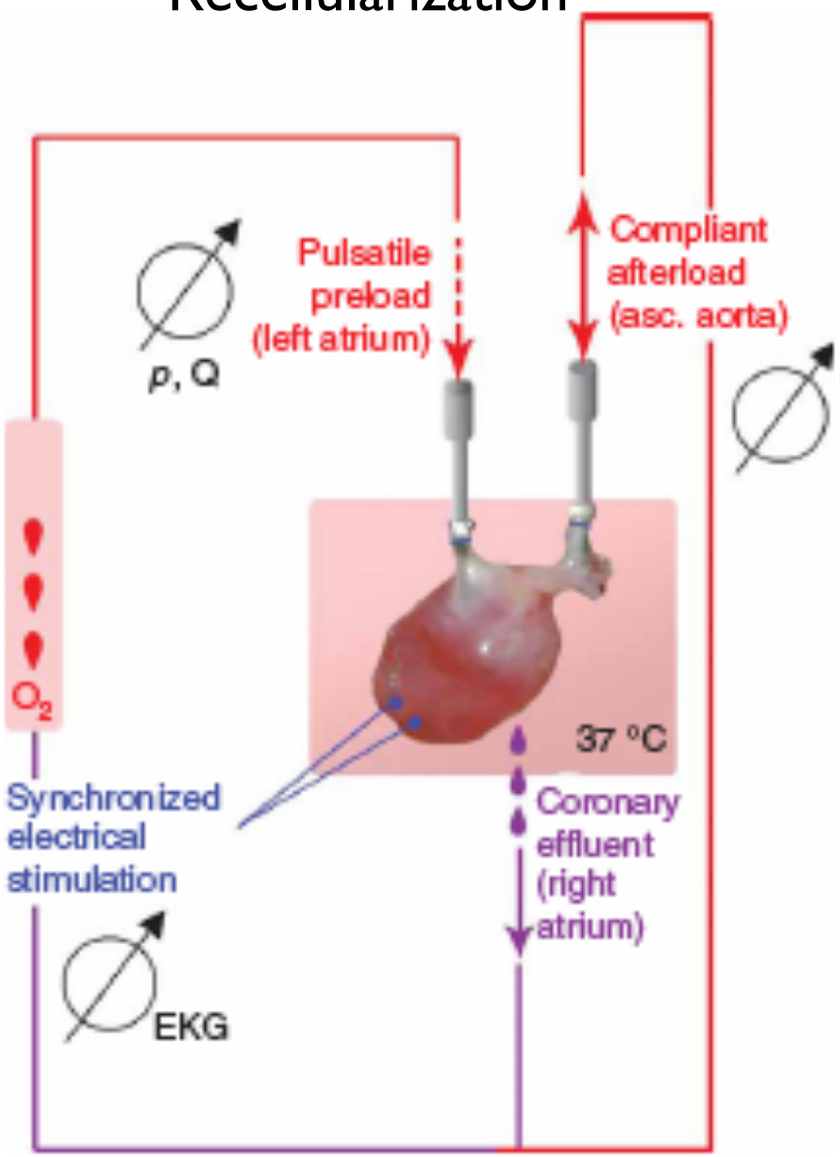
Bioreactor Model used for Recellularization

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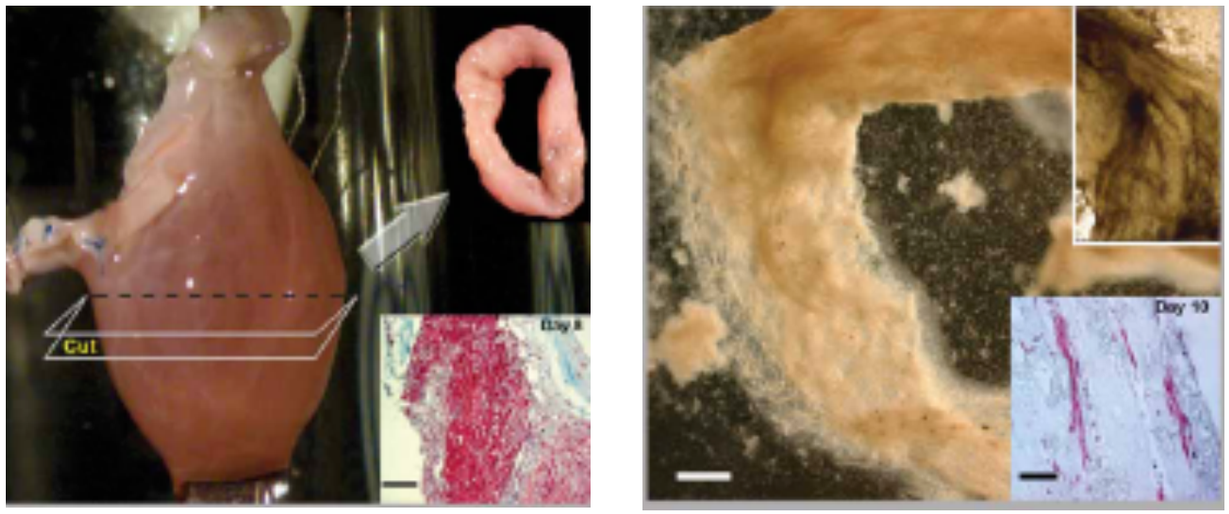
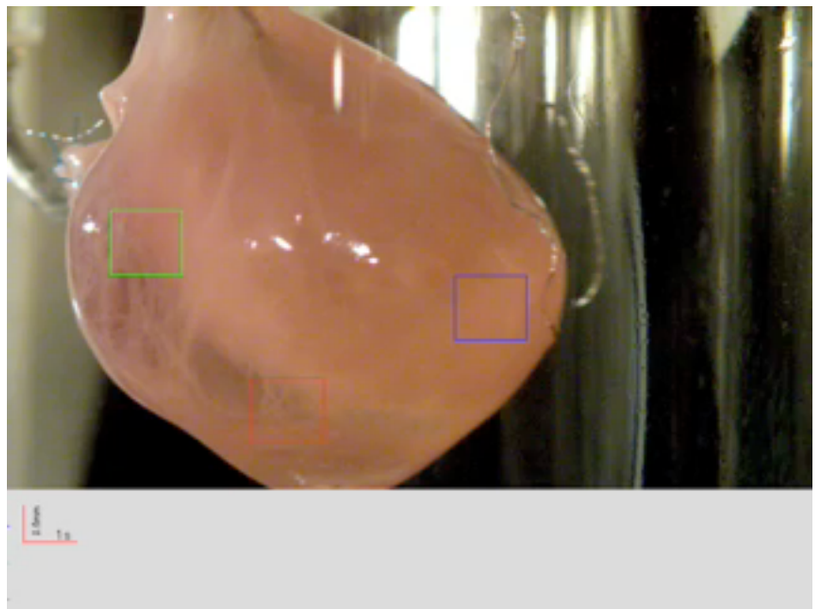


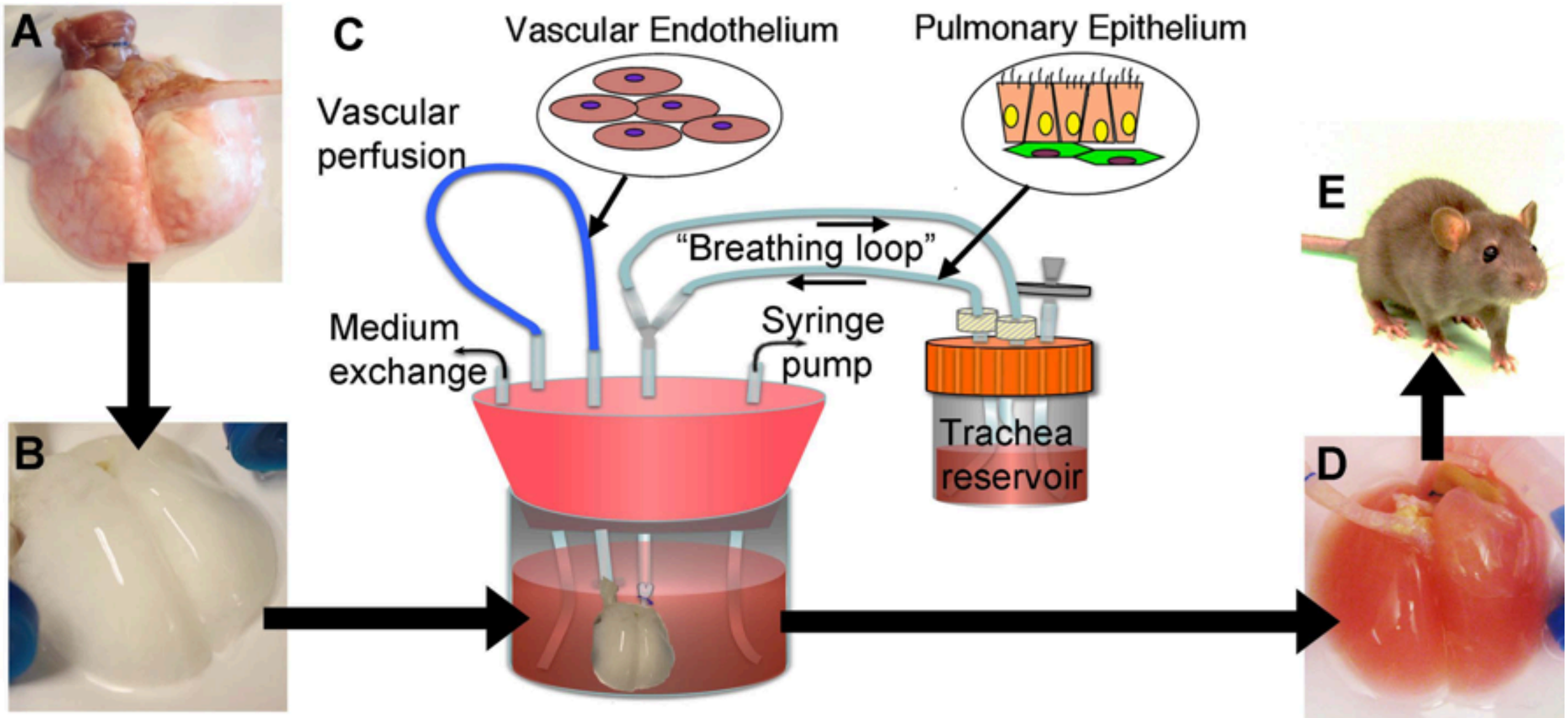



2% Contraction Fraction Compare to a Normal Heart

Ott HC, Taylor DA. Nat Med. 2008 Feb;14(2):213-21.

Sciencexpress Research Article

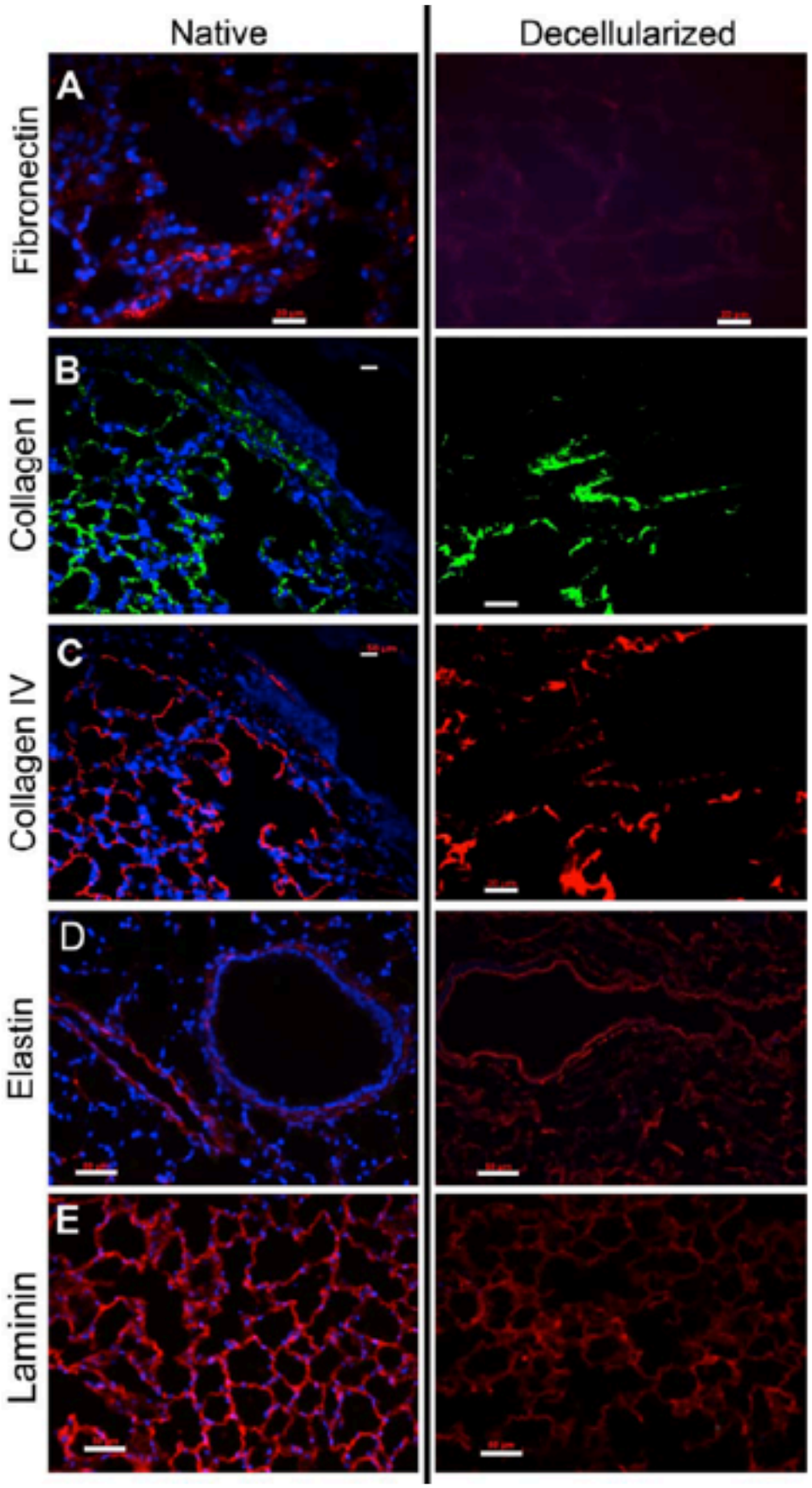
# Tissue-Engineered Lungs for in Vivo Implantation

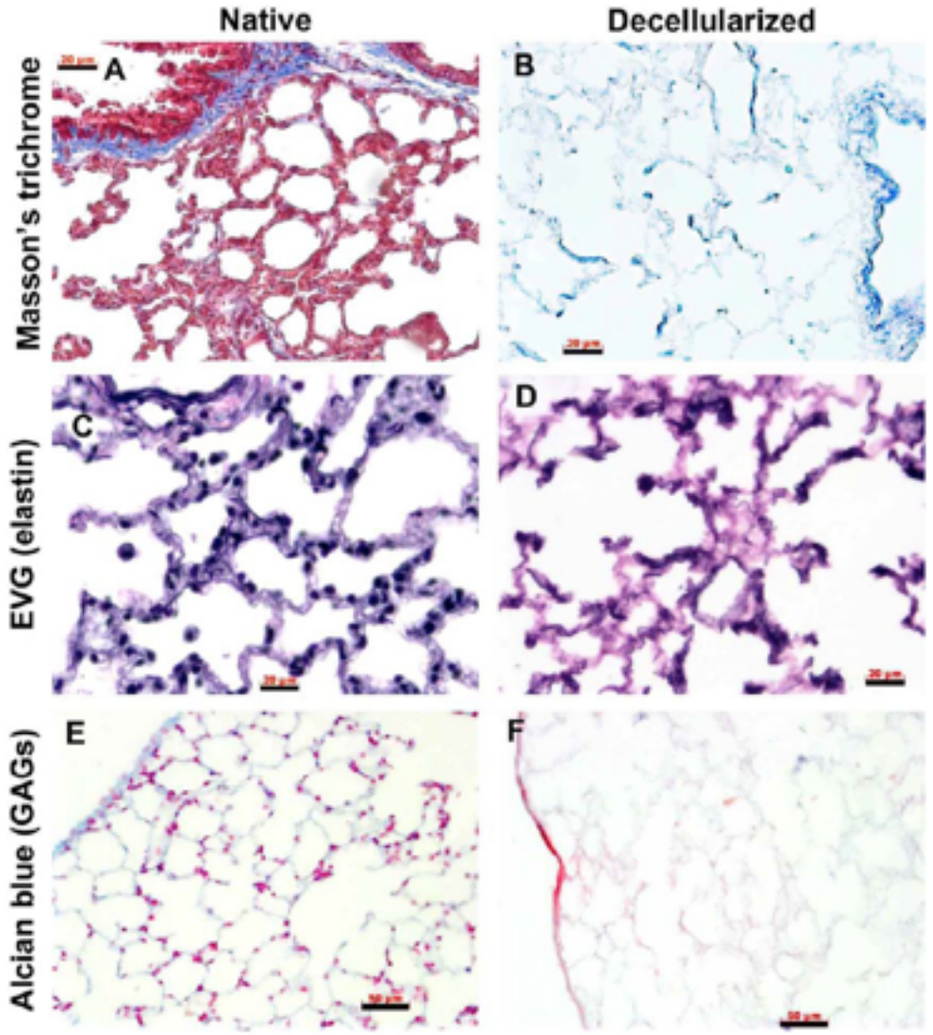


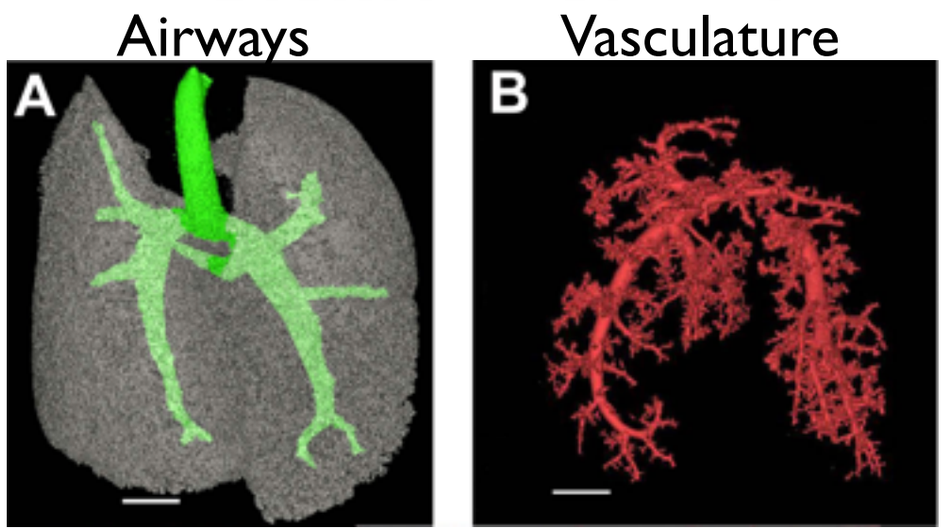

CHAPS (zwitterionic detergent)

Petersen TH, Niklason LE. Science. 2010 Jun 28.

# Characterization of Acellular Lung Matrix



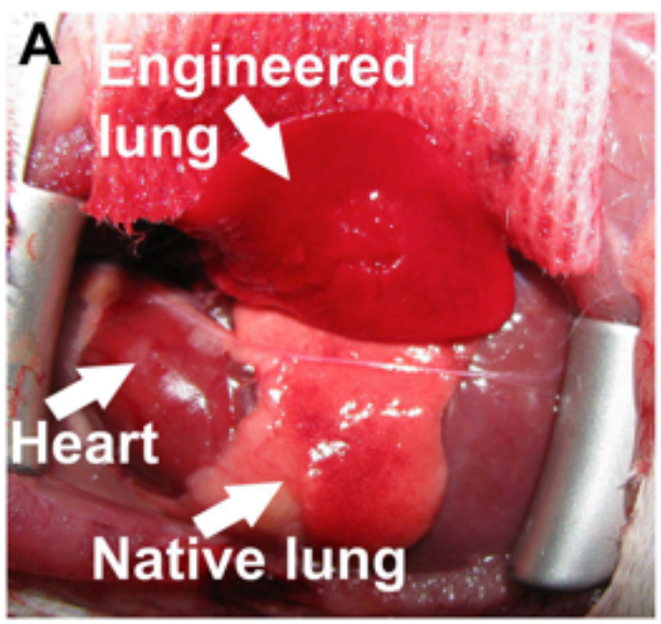



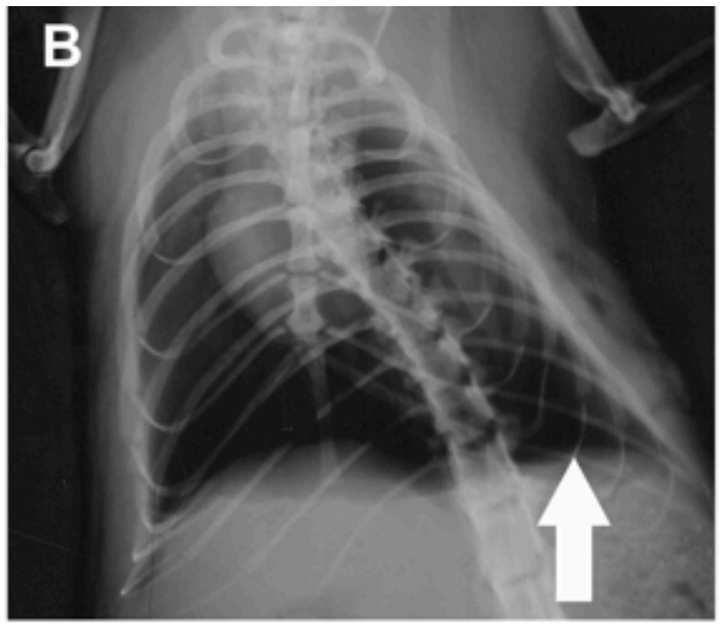



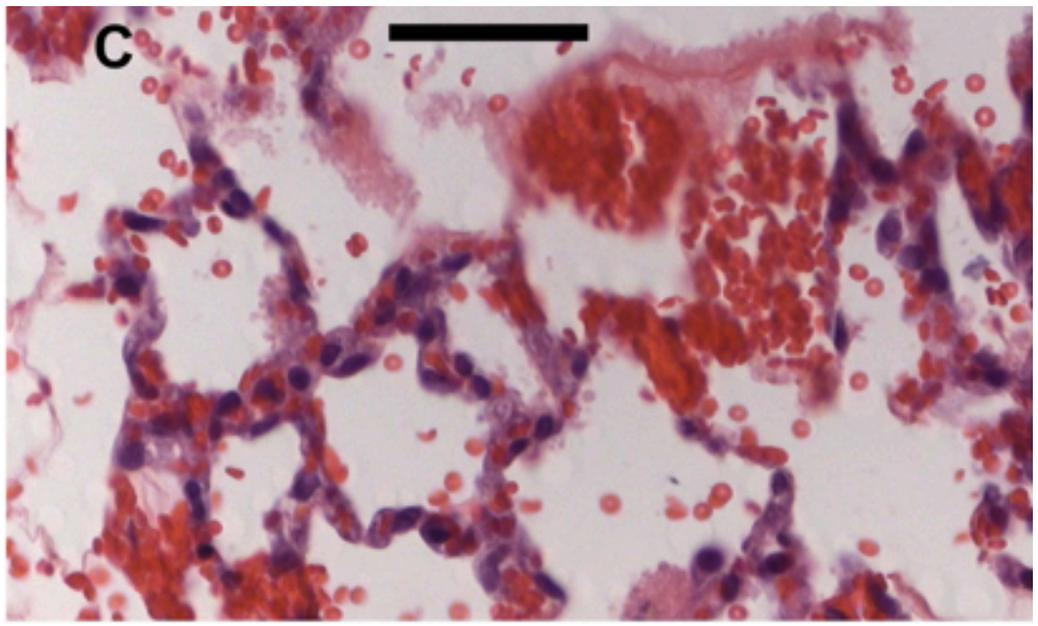

Micro-CT

Petersen TH, Niklason LE. Science. 2010 Jun 28.

# Implantation of Engineered Lungs into Rats



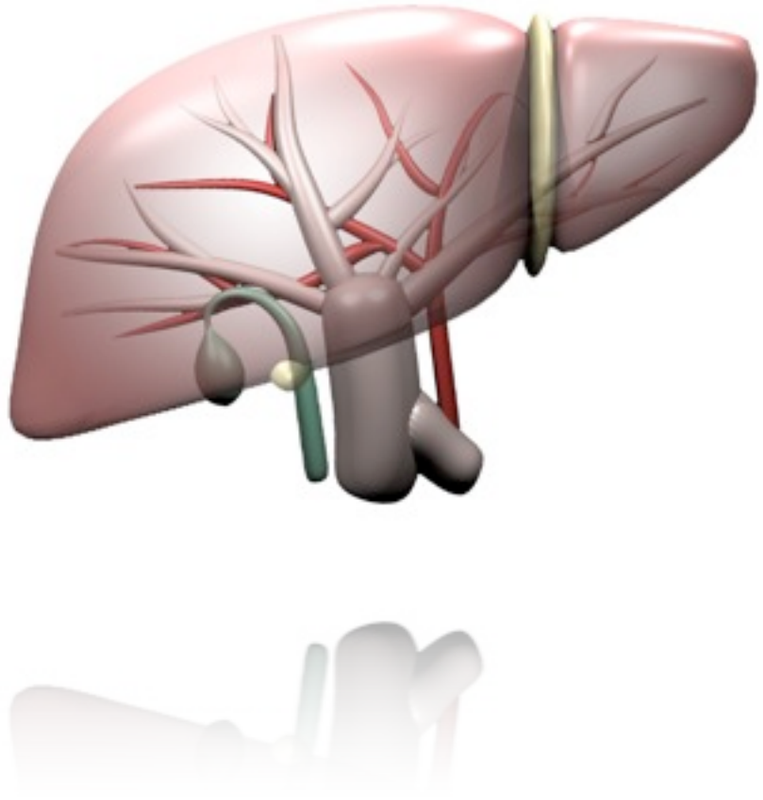




Implantation last from 45 min to 2h with effective O2 Exchange

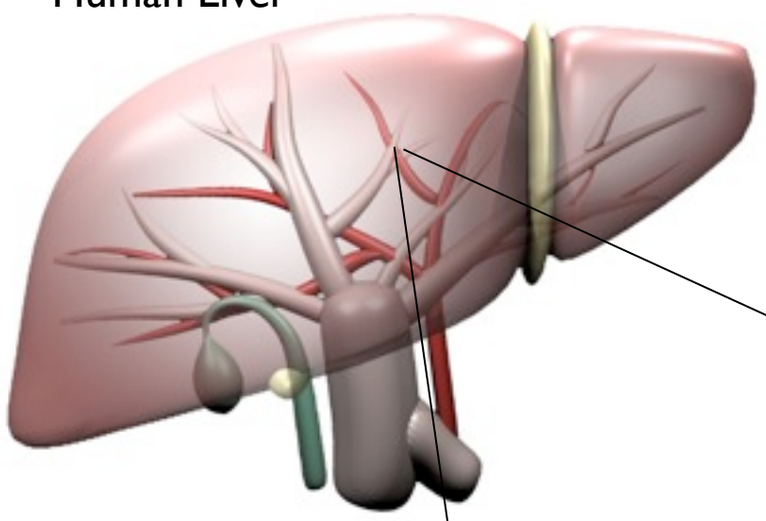
Petersen TH, Niklason LE. Science. 2010 Jun 28.

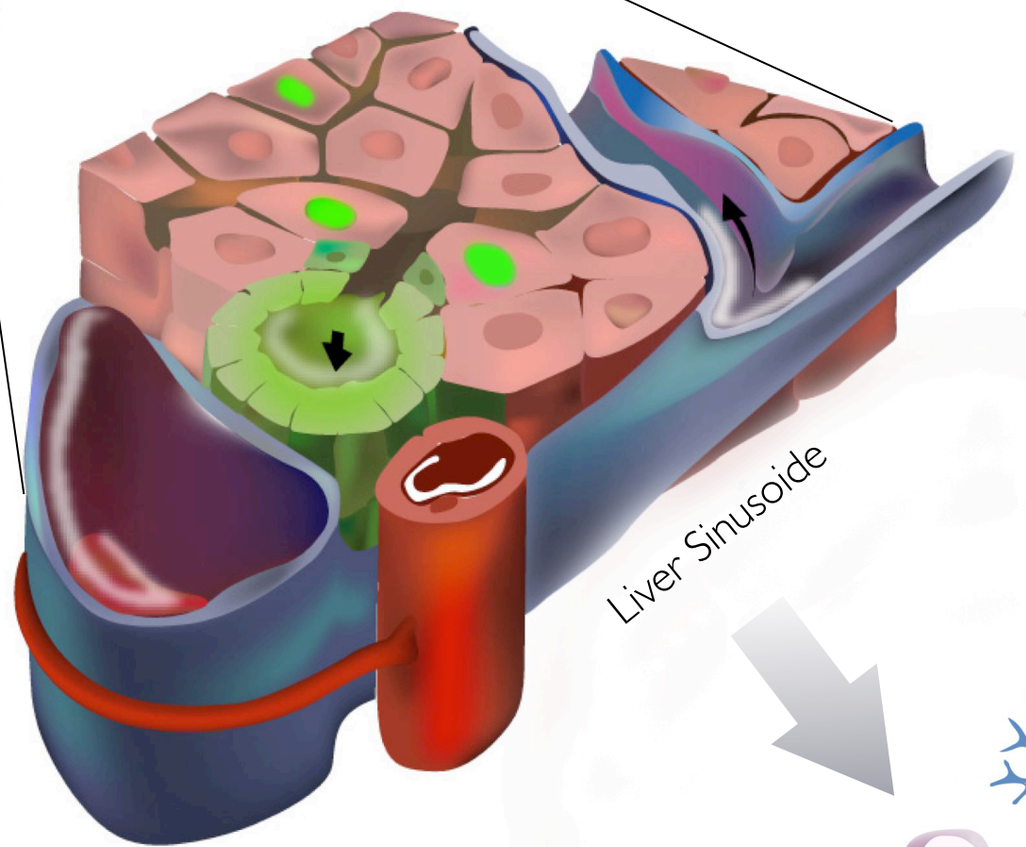
# Liver Bioengineering for Transplantation

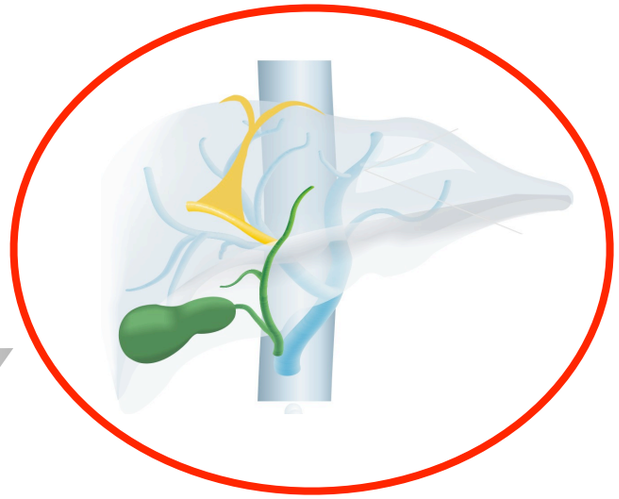


# Different Cellular and Acellular Components of the Liver

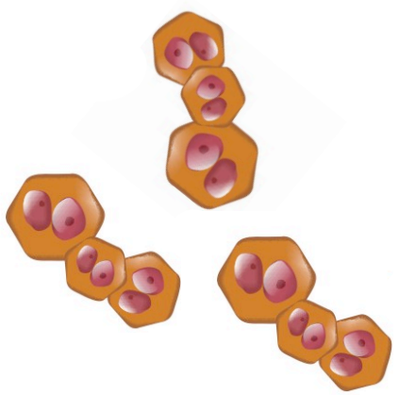
Human Liver



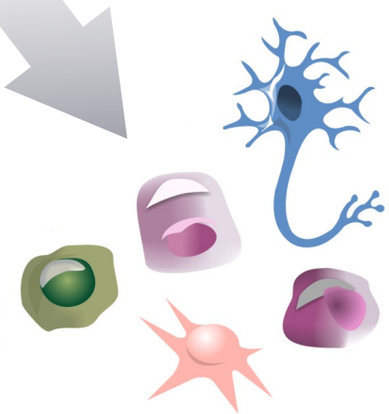


Extracellularmatrix
(ECM)

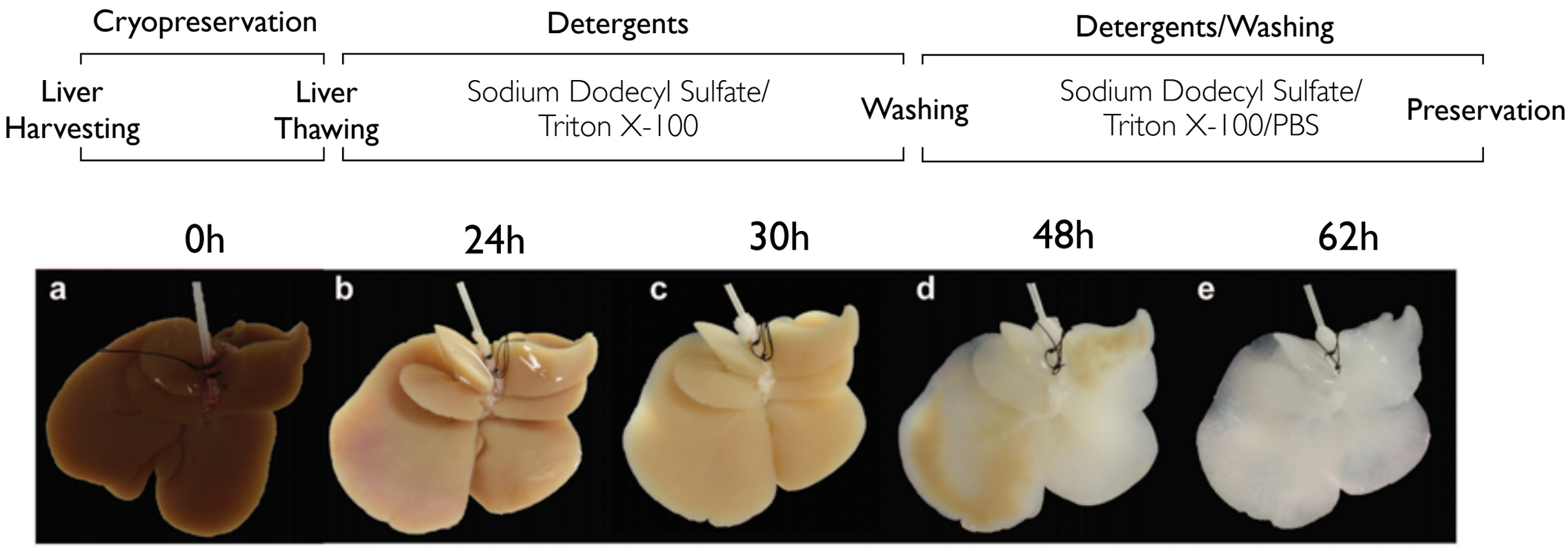


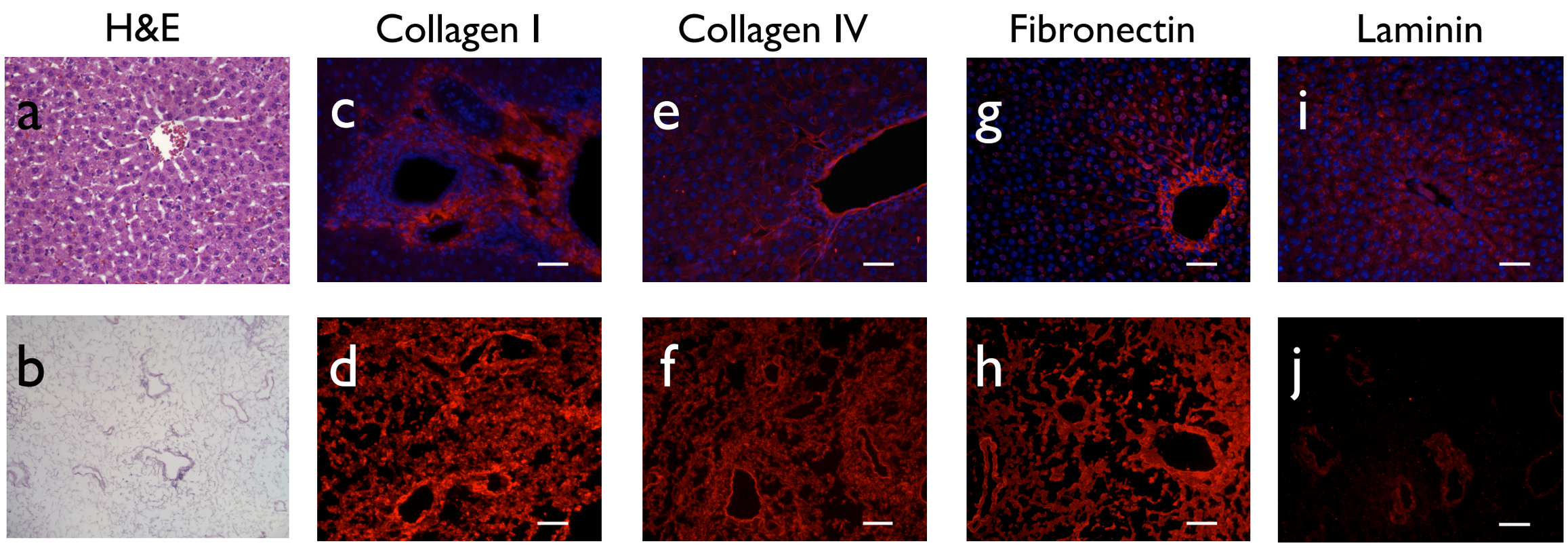
Parenchymal Liver Cells
(Hepatocytes)



Non-Parenchymal Liver Cells

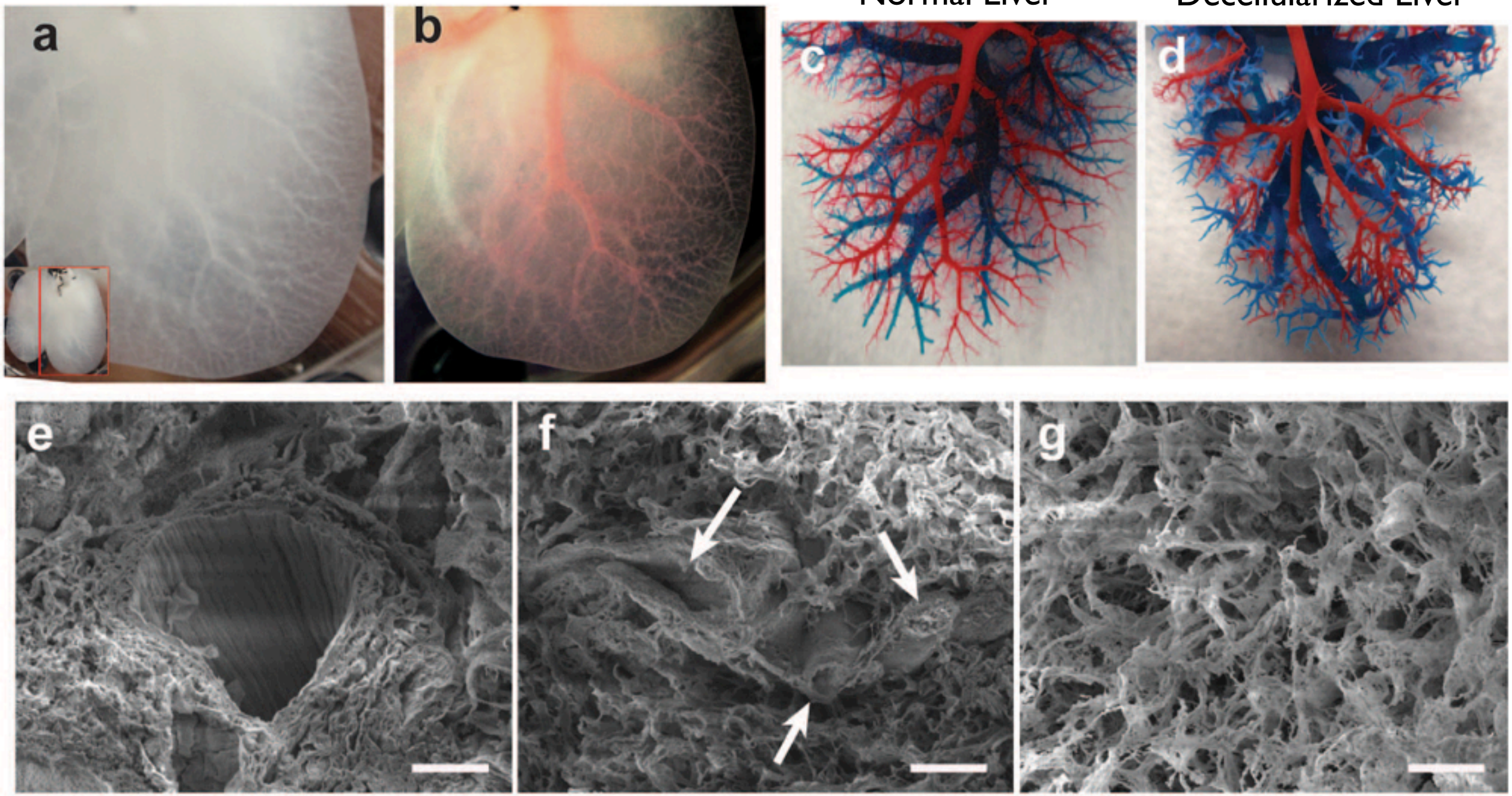
# Decellularization of Whole Livers



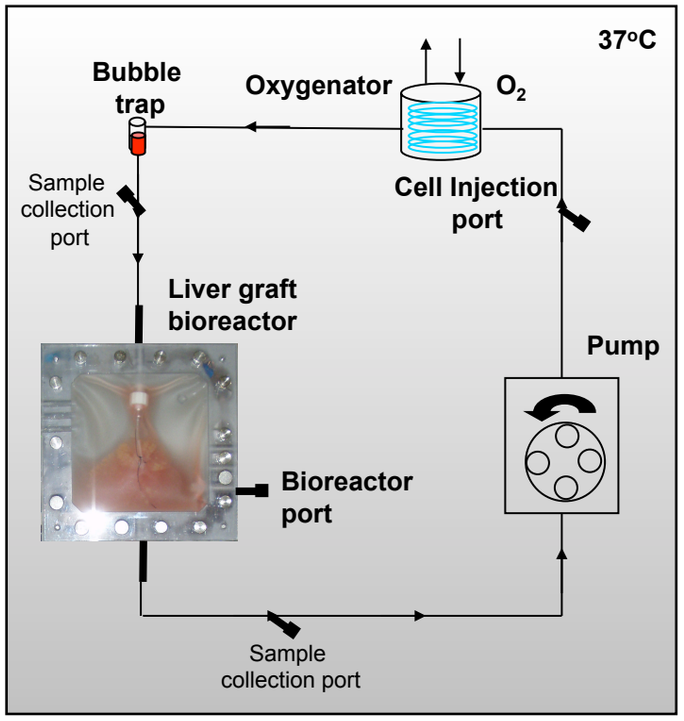

*Uygun B, Soto-Gutierrez A, et al. Nat Med. 2010;16(7):814-20.*

# Vascular Characterization and Preservation in the Natural Liver Scaffold

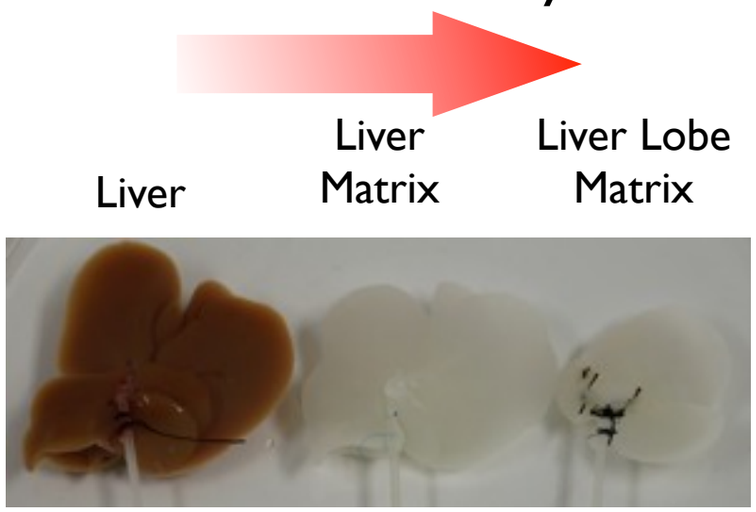


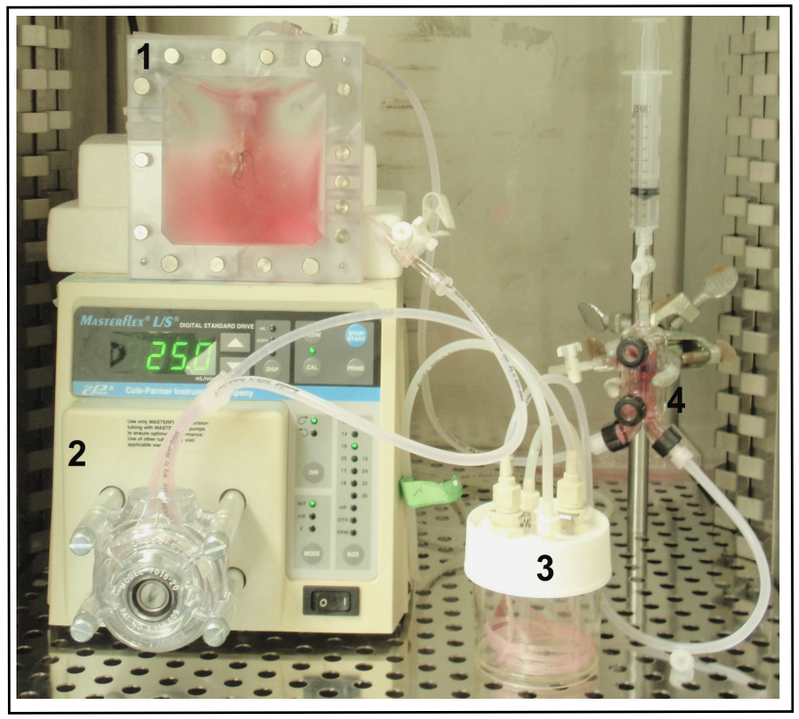

*Uygun B, Soto-Gutierrez A, et al. Nat Med. 2010;16(7):814-20.*

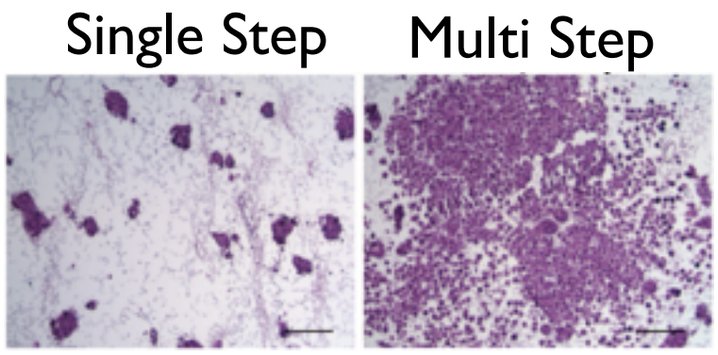
# Re-engineering the Liver

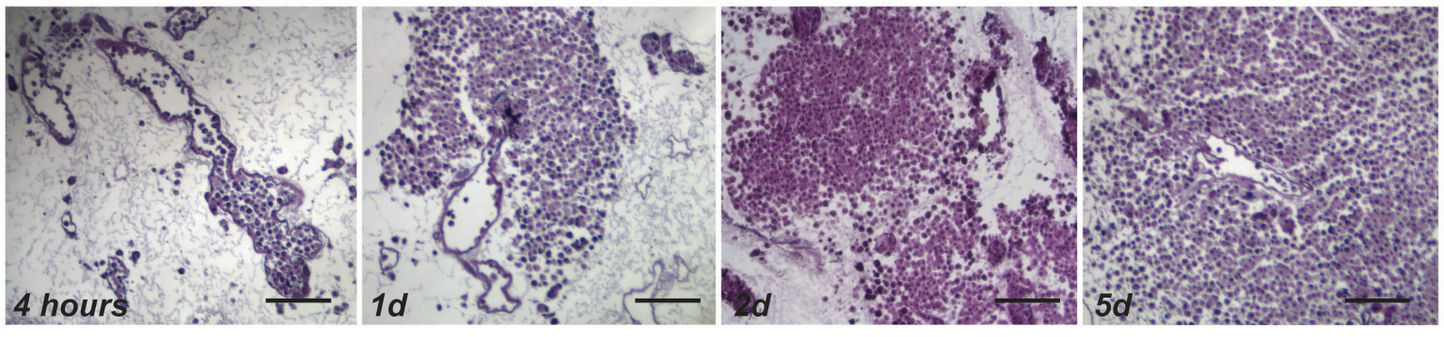



Recellularization System

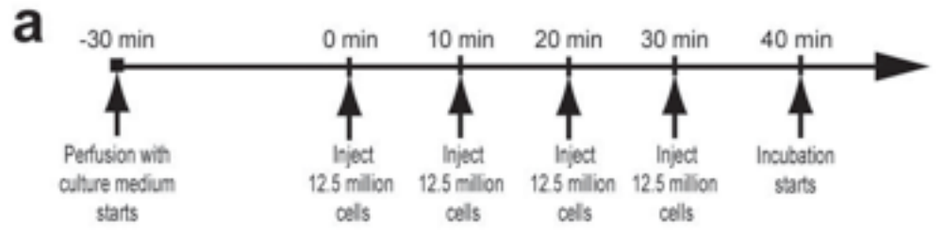


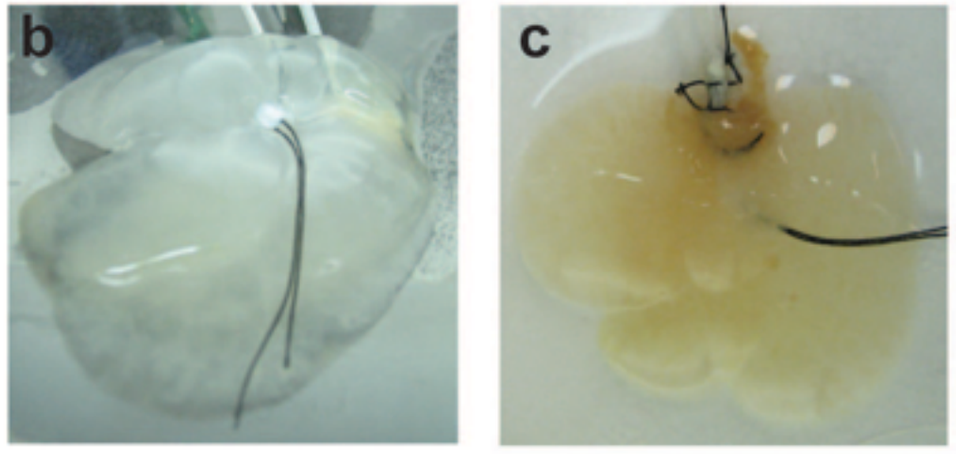




Hepatocyte Integration and Engraftment over Time
(~90% of Cell Engraftment)



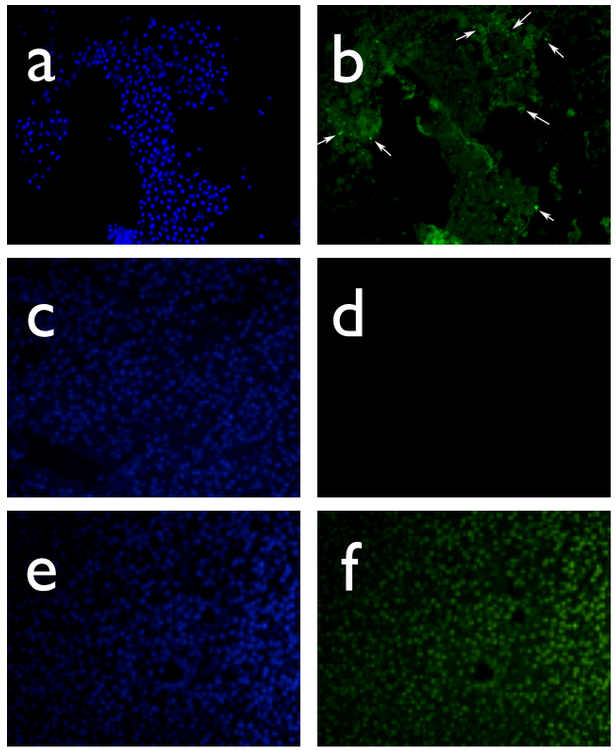



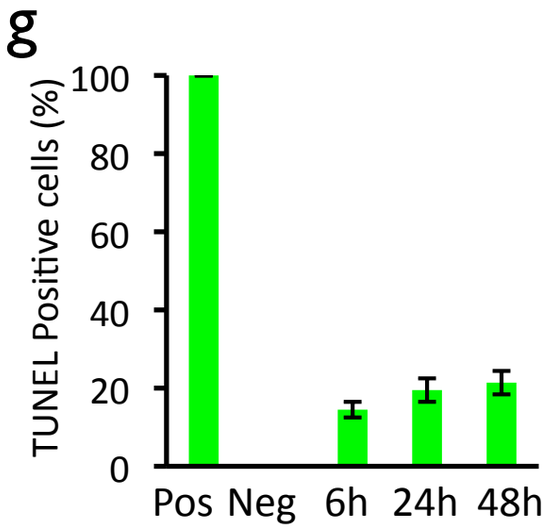

Reconstitution of about 10% of total cell mass

*Uygun B, Soto-Gutierrez A, et al. Nat Med. 2010;16(7):814-20.*

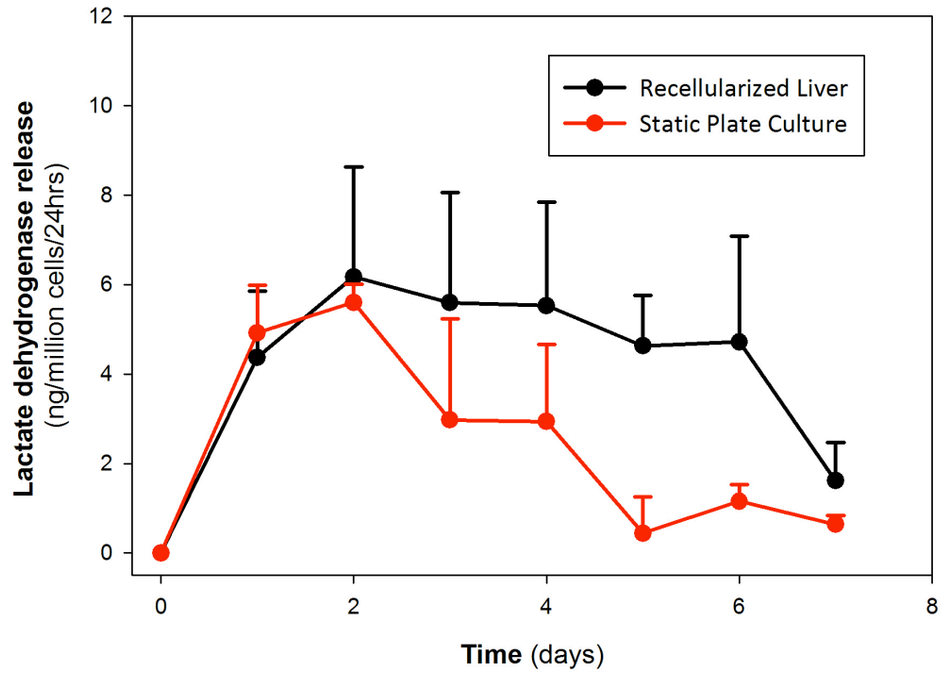
# Characterization of the Re-engineered Livers

## Tunnel Staining

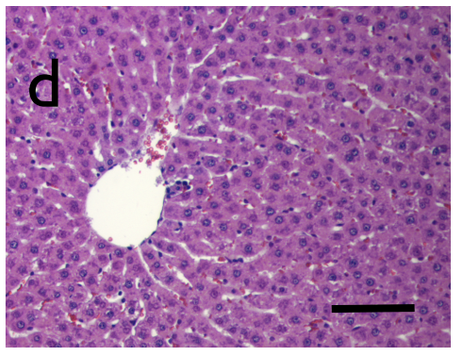


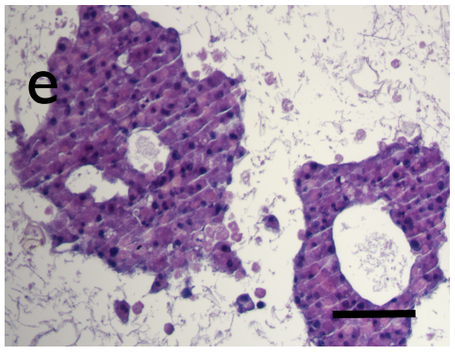




## LDH

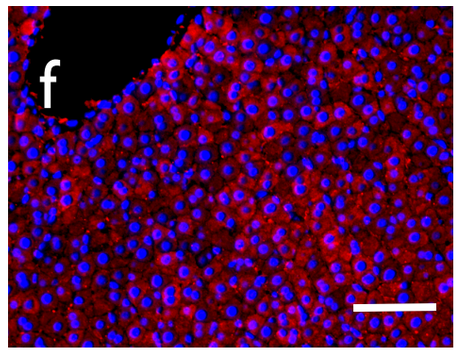


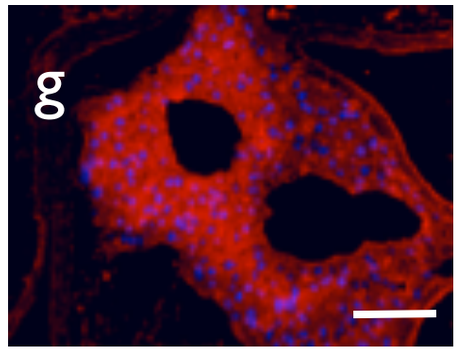

## H&E



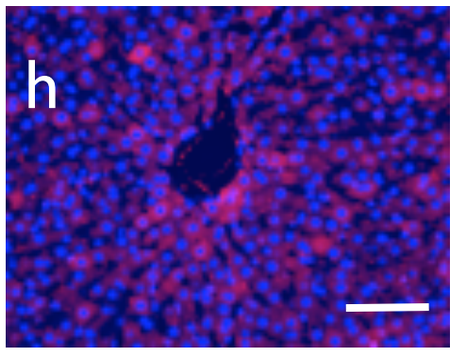



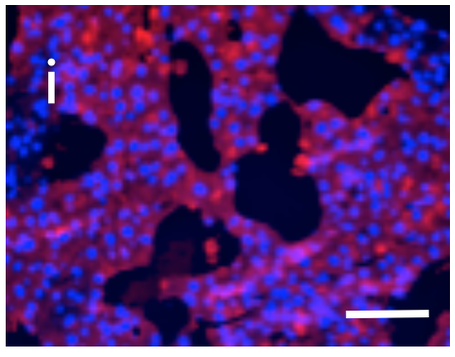

## ALB



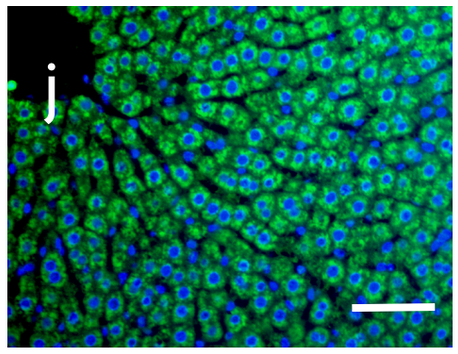



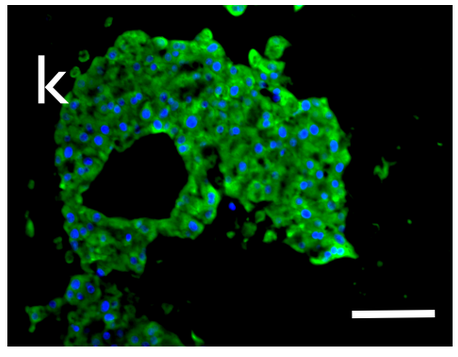

## G6Pase



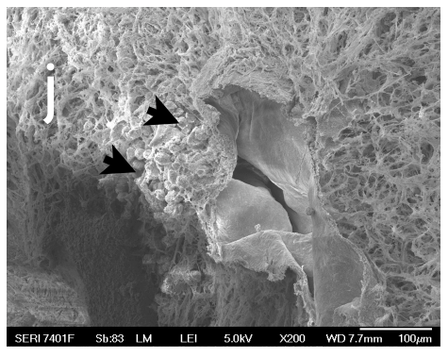



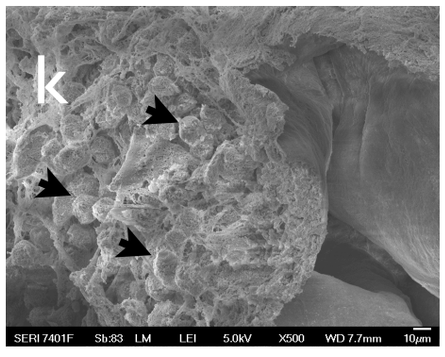

## UGT1A




## SEM

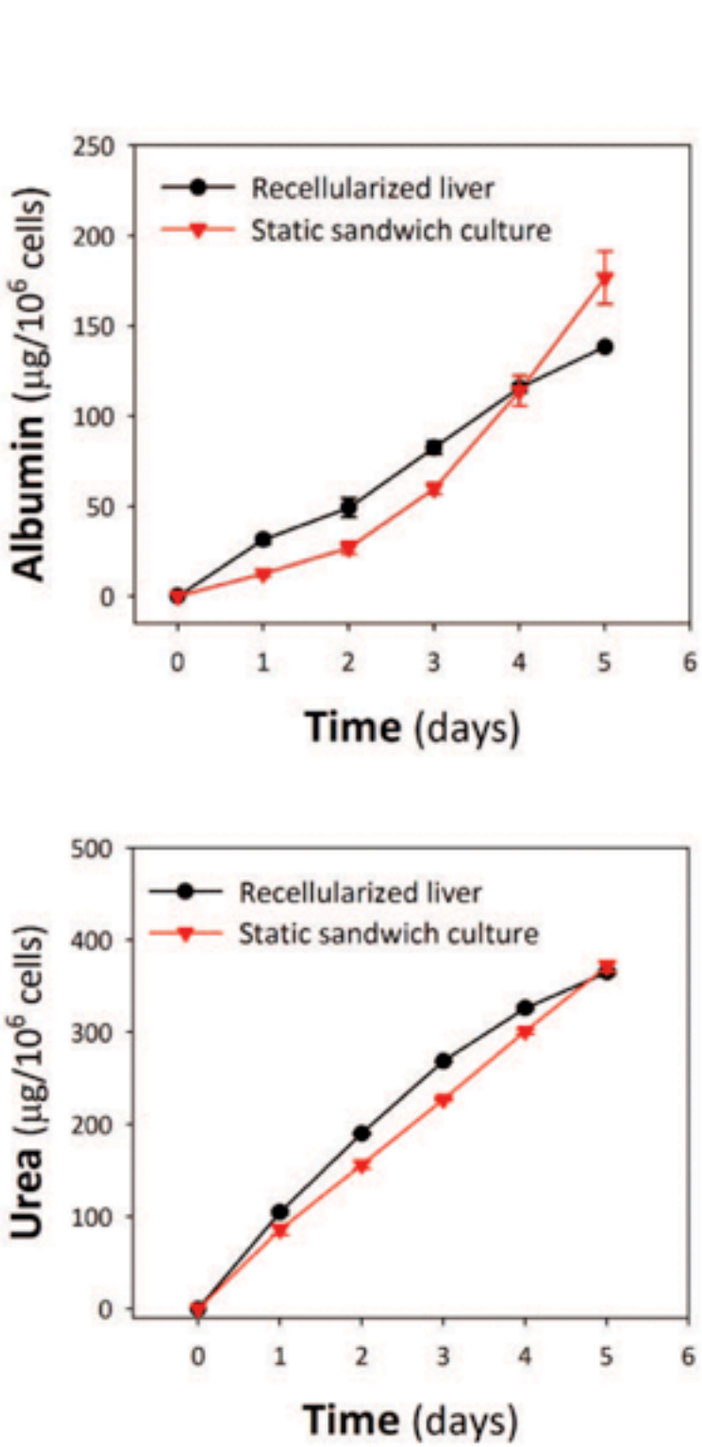


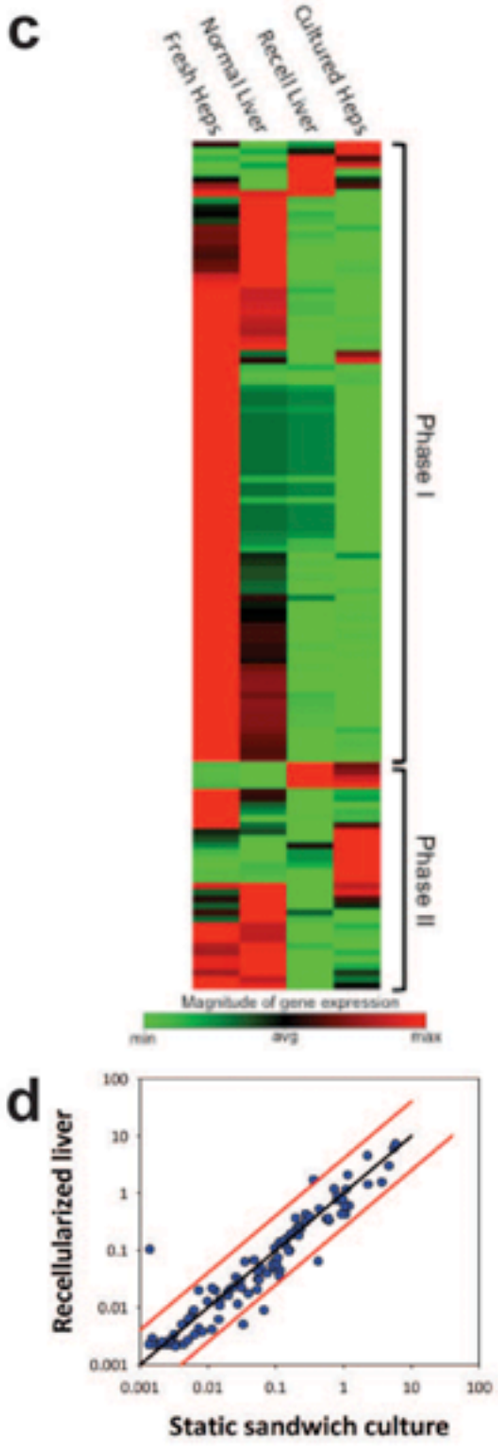



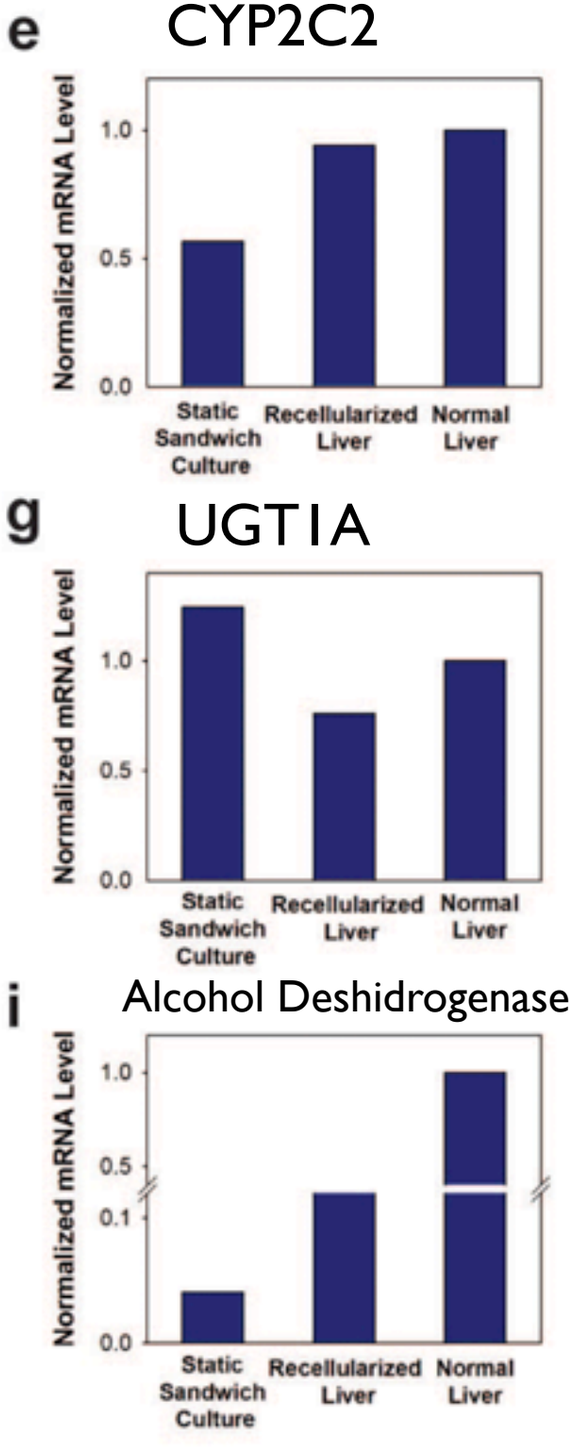

*Uygun B, Soto-Gutierrez A, et al. Nat Med. 2010;16(7):814-20.*

# Functional Characterization of Re-engineered Livers in the Organ Culture System

Gene Array (Drug Metabolism)

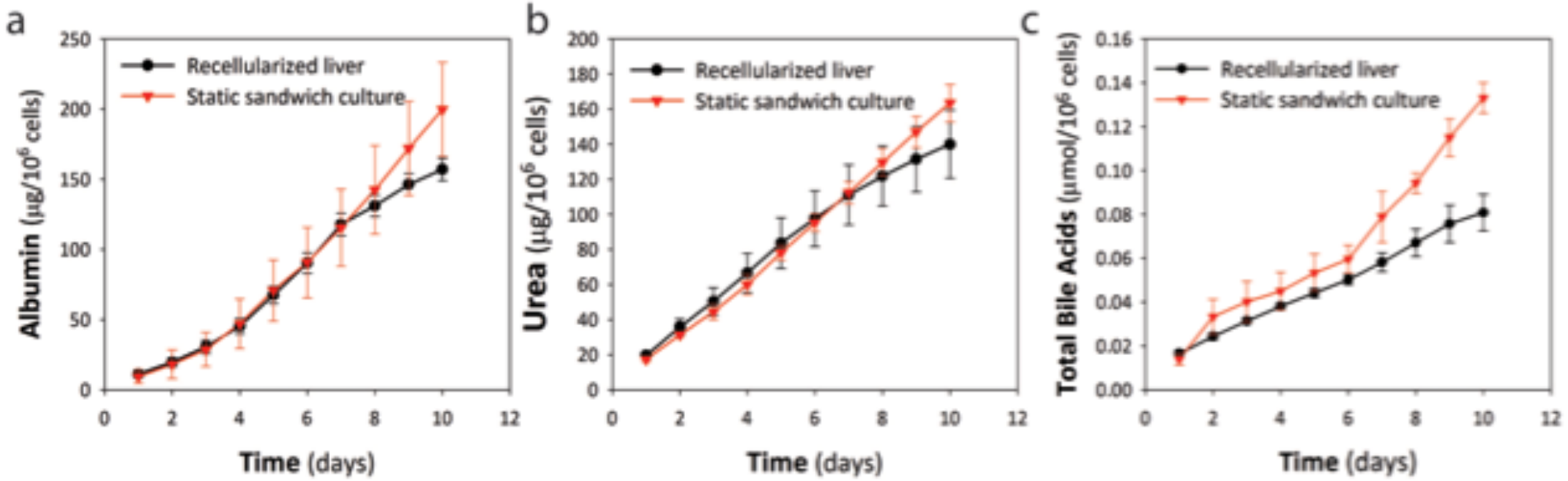


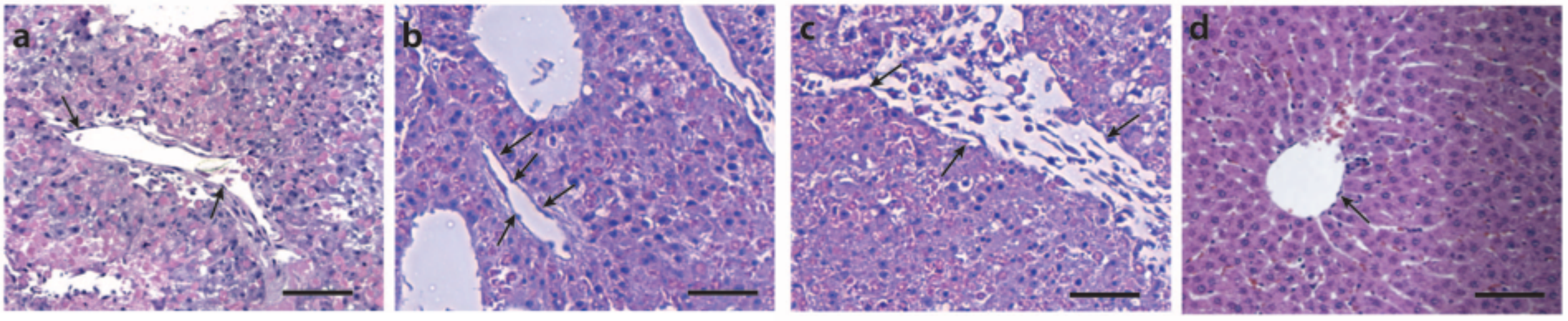

*Uygun B, Soto-Gutierrez A, et al. Nat Med. 2010;16(7):814-20.*

# Functional Analysis of Massive Re-cellularization of Liver Grafts



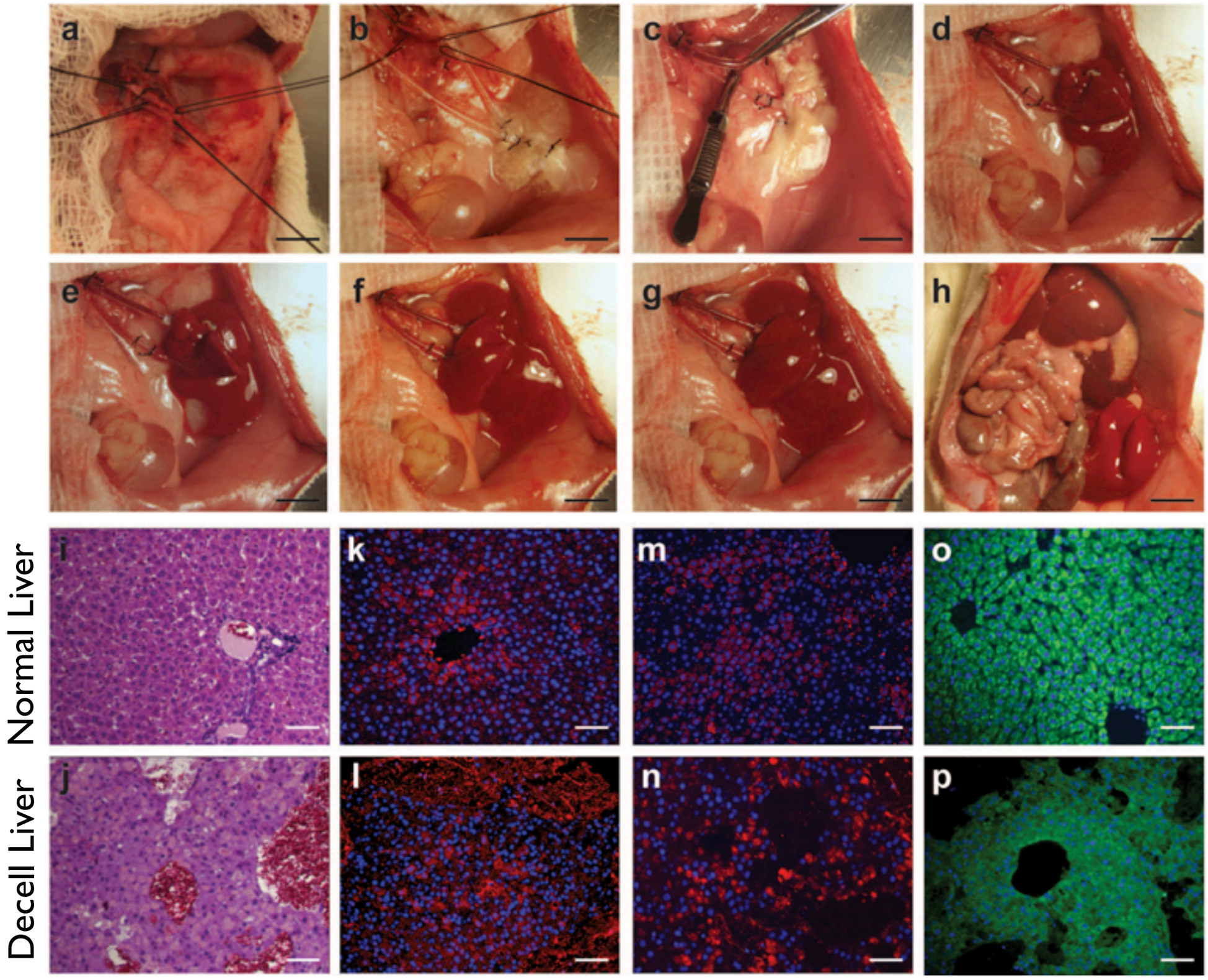

## Reconstitution of about 50% of total cell mass

Normal Liver




*Uygun B, Soto-Gutierrez A, et al. Nat Med. 2010;16(7):814-20.*

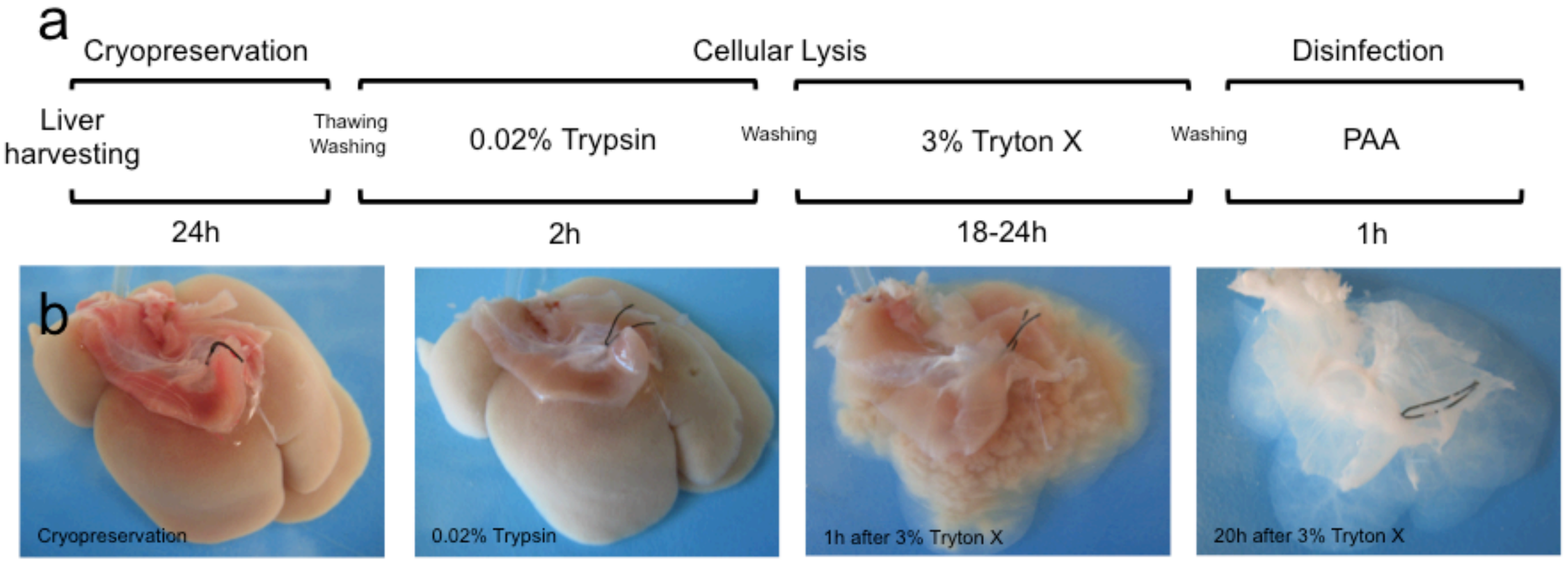
# In vivo Implantation of the Re-engineered Liver Grafts




Implantation lasted up to 8h!!!!!

*Uygun B, Soto-Gutierrez A, et al. Nat Med. 2010;16(7):814-20.*

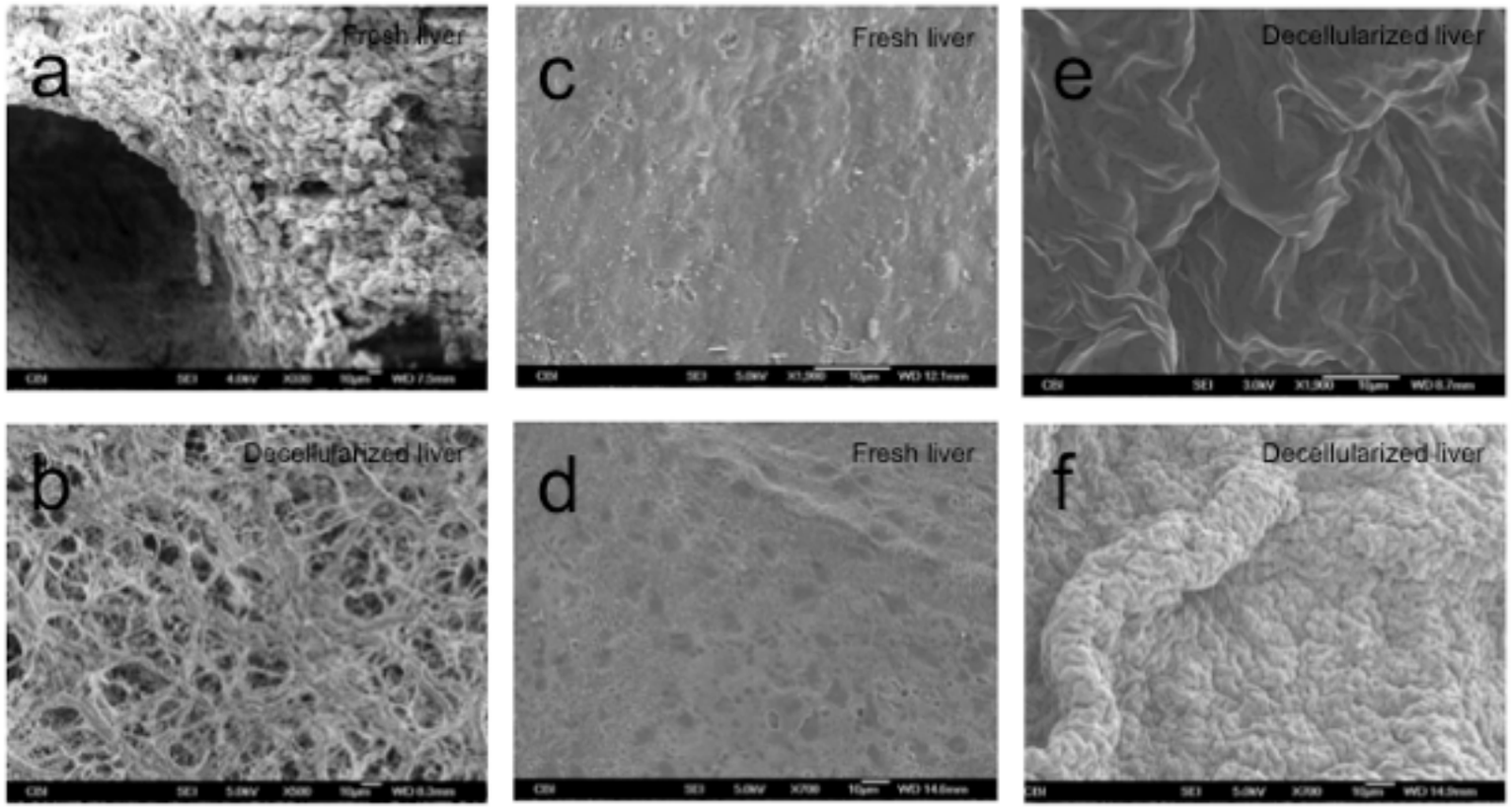
# Improved Whole Liver Decellularization Protocol



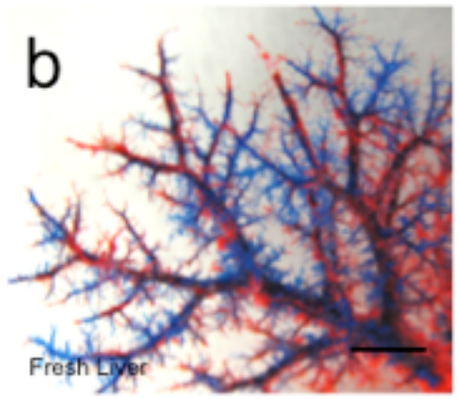

*Soto-Gutierrez A, et al.* Tissue Eng Part; 2011 Mar 4.

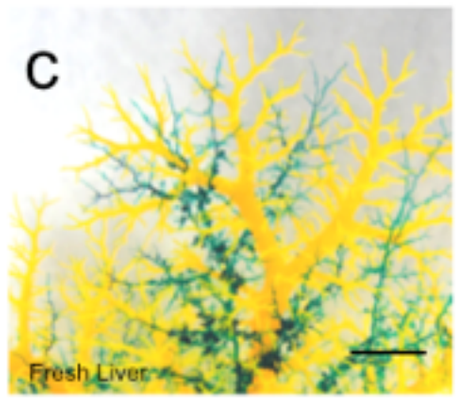
# Characterization of the Decellularized Liver

SEM (Glisson's Capsule)

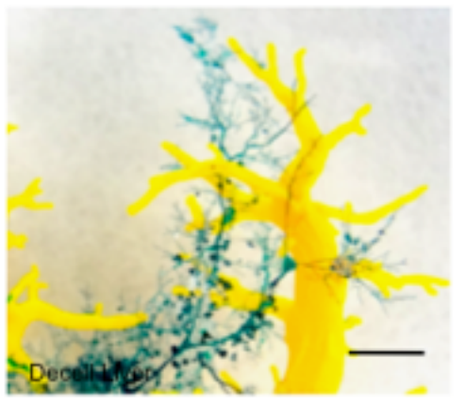



Fresh Liver



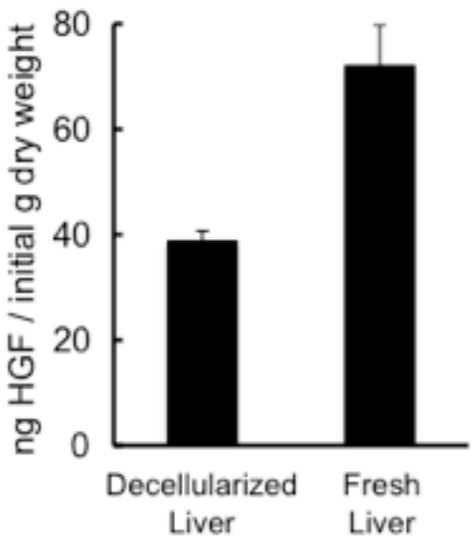




Decell Liver

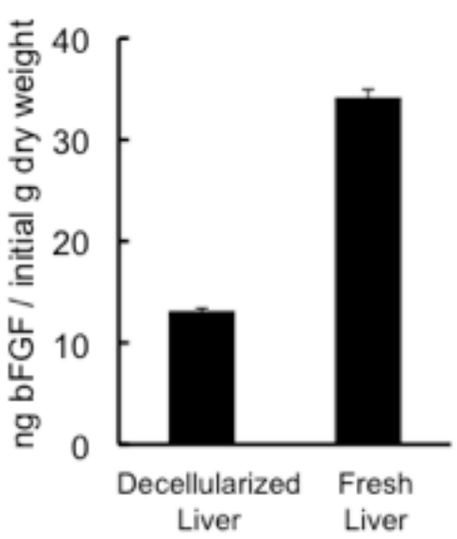



Hepatic Artery  Portal Vein  Bile Duct  Central Vein

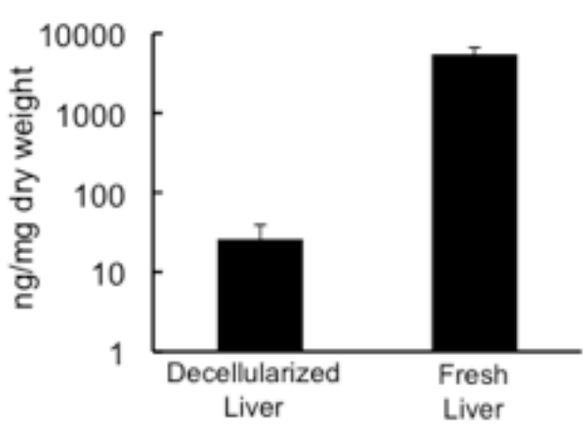
## HGF Content

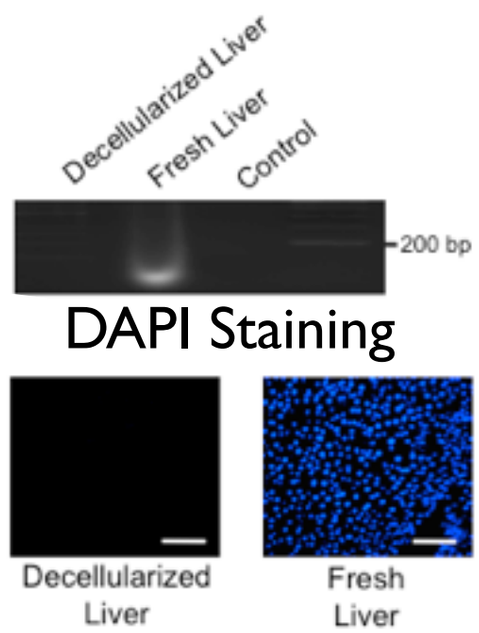



## FGF Content




## DNA Content

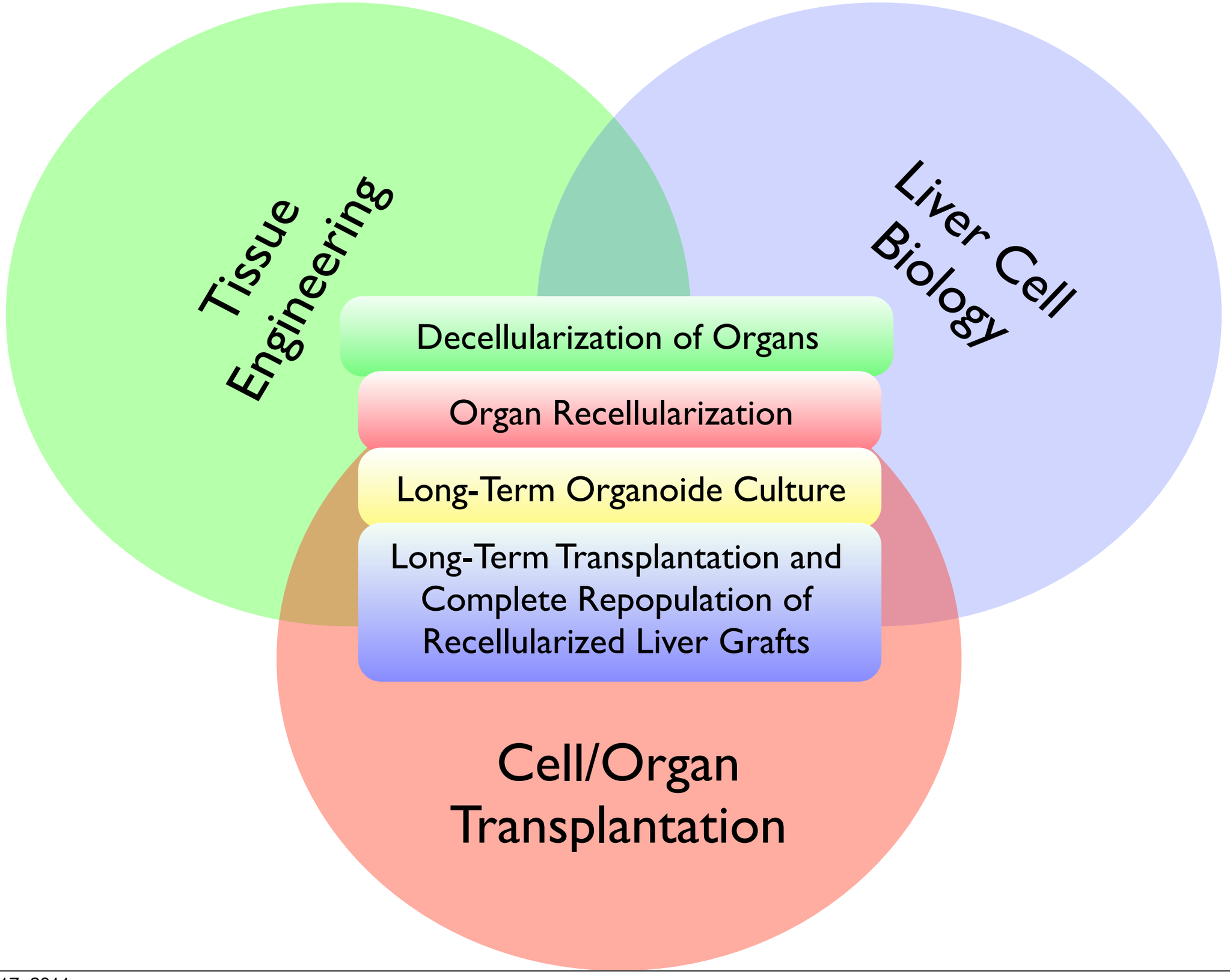


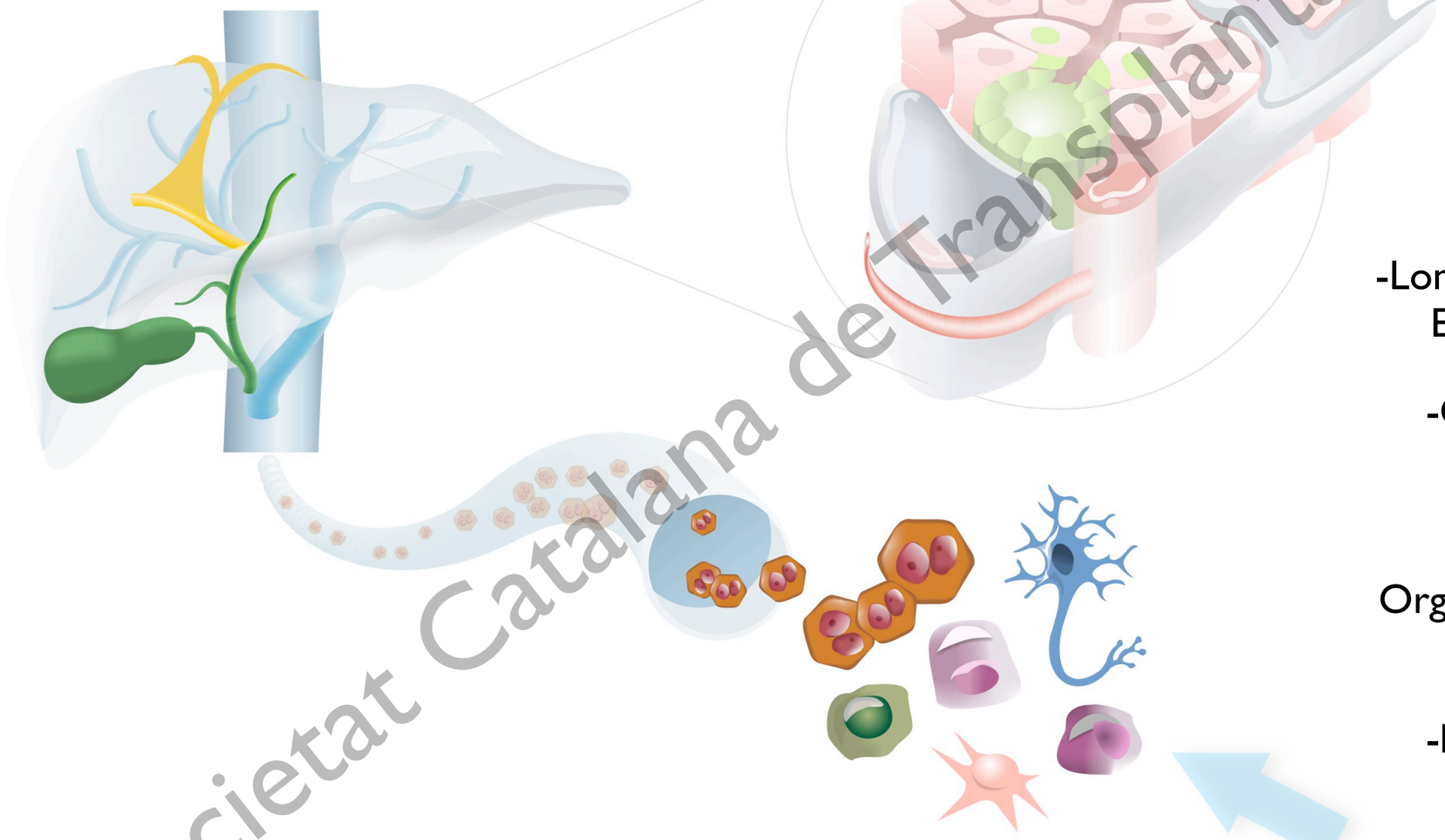

*Soto-Gutierrez A, et al.* Tissue Eng Part; 2011 Mar 4.

# Bioengineering in Organ Transplantation:

## *A Regenerative Medicine Approach*



# Organ Engineering Using Cell Transplantation and Tissue Engineering Principles



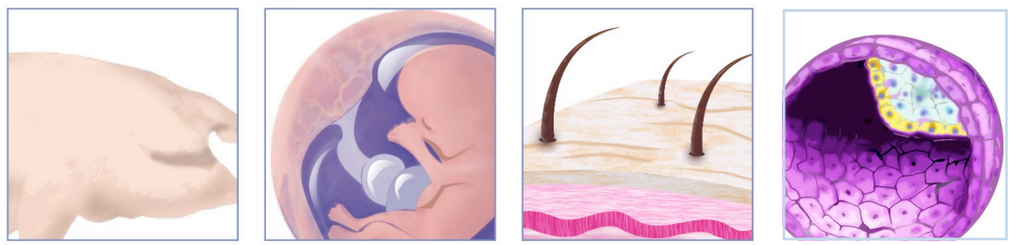
Re-engineering of Complex Hepatic Structures

-Long-Term Transplantation of Engineered Liver Grafts

-Cell Differentiation and Interaction Studies

-New Generation of Organoids for Extracorporeal Support

-Bile Duct Regeneration



Pig Cells | Fetal Cells | iPS Cells | ES Cells


Soto-Gutiérrez A, Kobayashi N, et al. Nat Biotechnol. 2006;24(11):1412-9.
Basma H, Soto-Gutierrez A, et al. Gastroenterology. 2009 Mar;136(3):990-9.
Uygun B, Soto-Gutierrez A, et al. Nat Med. 2010 Jul;16(7):814-20.

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Strom's Lab
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Hiroshi Yagi

**Center for Engineering in Medicine**

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Massachusetts General Hospital
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