UCSF Department of Surgery

Tissue Engineering in Space

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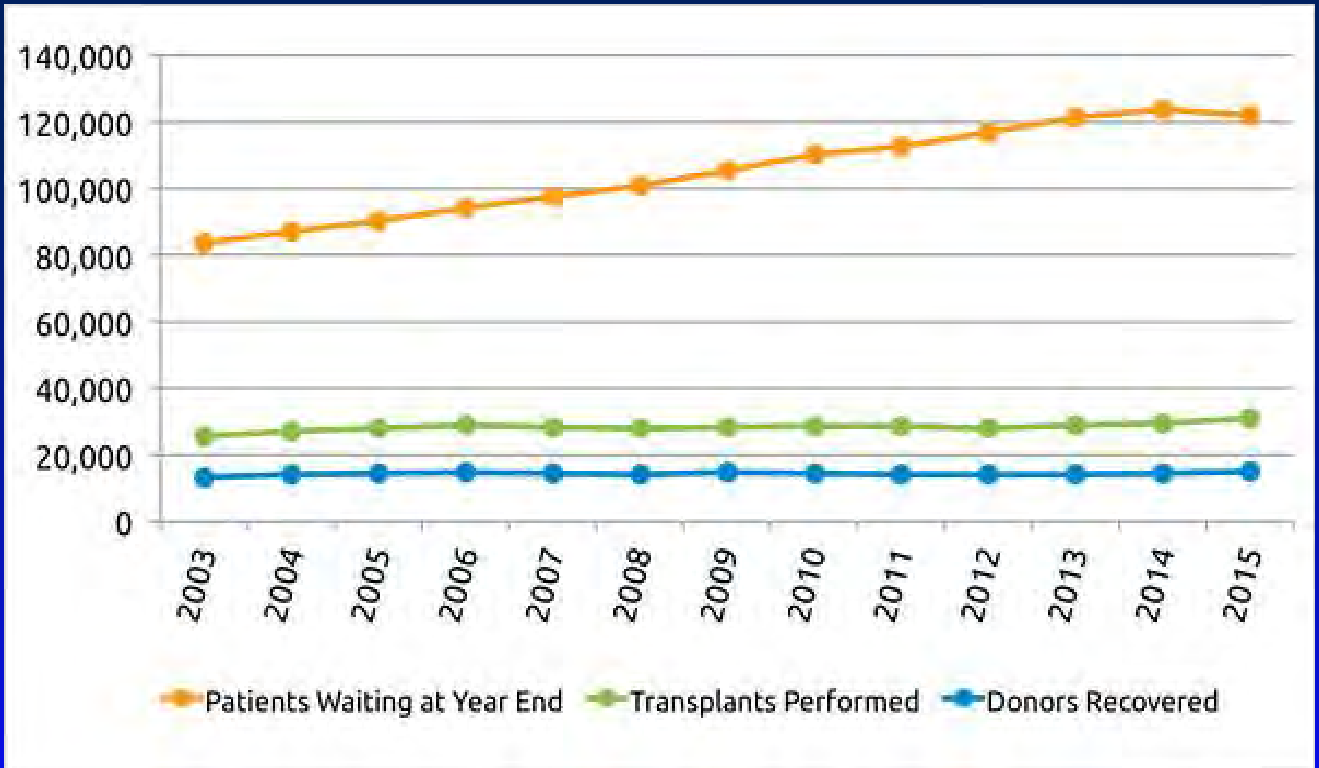
Osher Mini Medical School
Space Medicine and Its Influence on Earth: Out of this World Healthcare
March 3, 2021

Agenda

- Why tissue engineering
- What is the current state of the art
- Why tissue engineering in space makes sense

End-stage Organ Failure – Donor Organ Availability is a Major Limitation of Transplantation



optn.transplant.hrsa.gov

Evolution of Surgery

- Resection / Repair
- Reconstruction
- Transplantation
- Regeneration

Joseph E. Murray, M.D.
- 1990 Nobel Laureate in Medicine
- Performed 1st successful human kidney transplant in 1954

Vital Organs and Assist Devices

- Heart – Left Ventricular Assist Device (LVAD)
- Lung – Extracorporeal Membrane Oxygenation (ECMO)
- Kidney – Hemodialysis
- Liver - ?

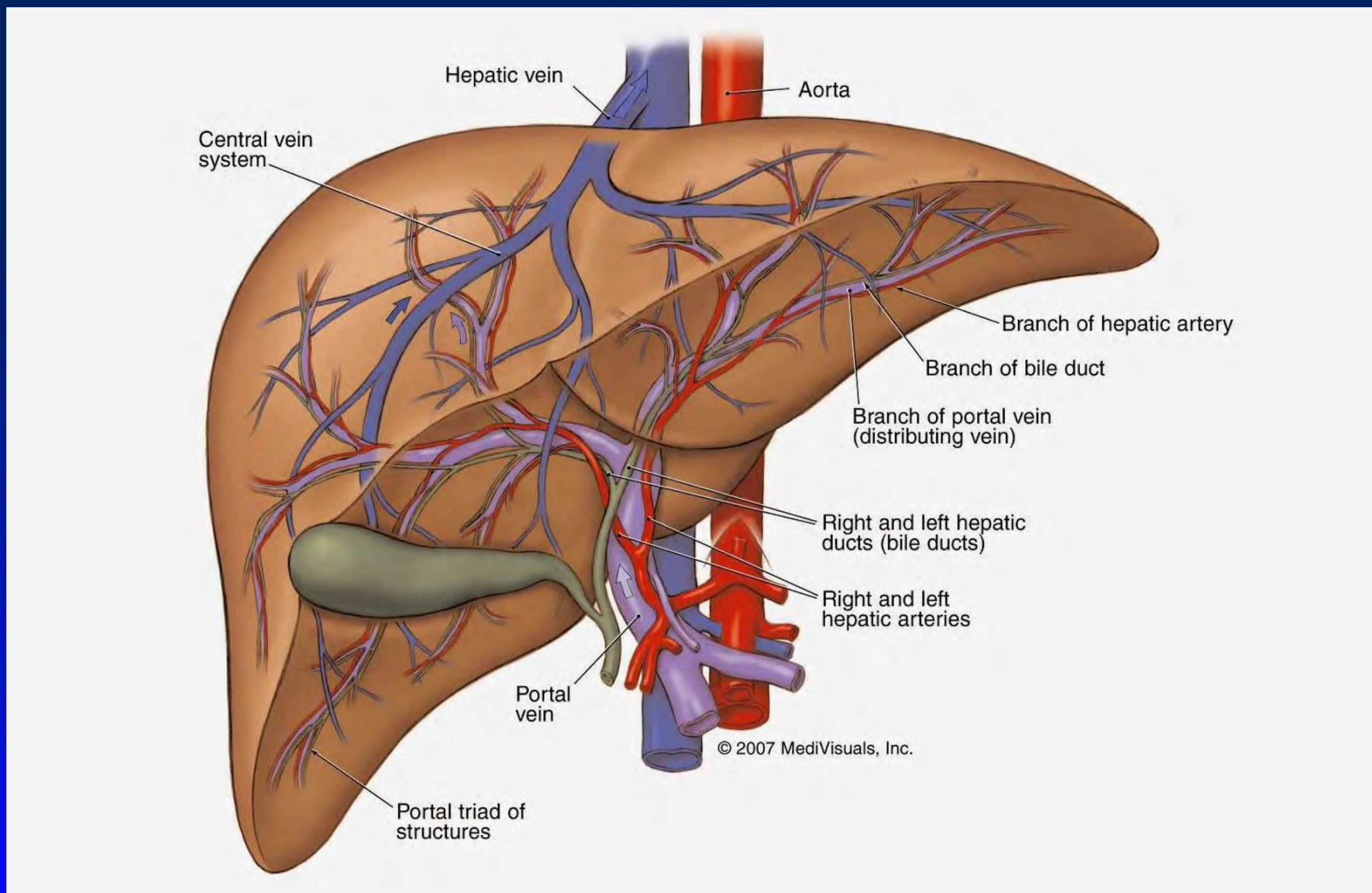
Liver Functions

- Central regulator of whole body metabolism and energy balance (glucose and lipid)
- Drug and toxin metabolism
- Clears bilirubin and makes bile
- Serum protein synthesis

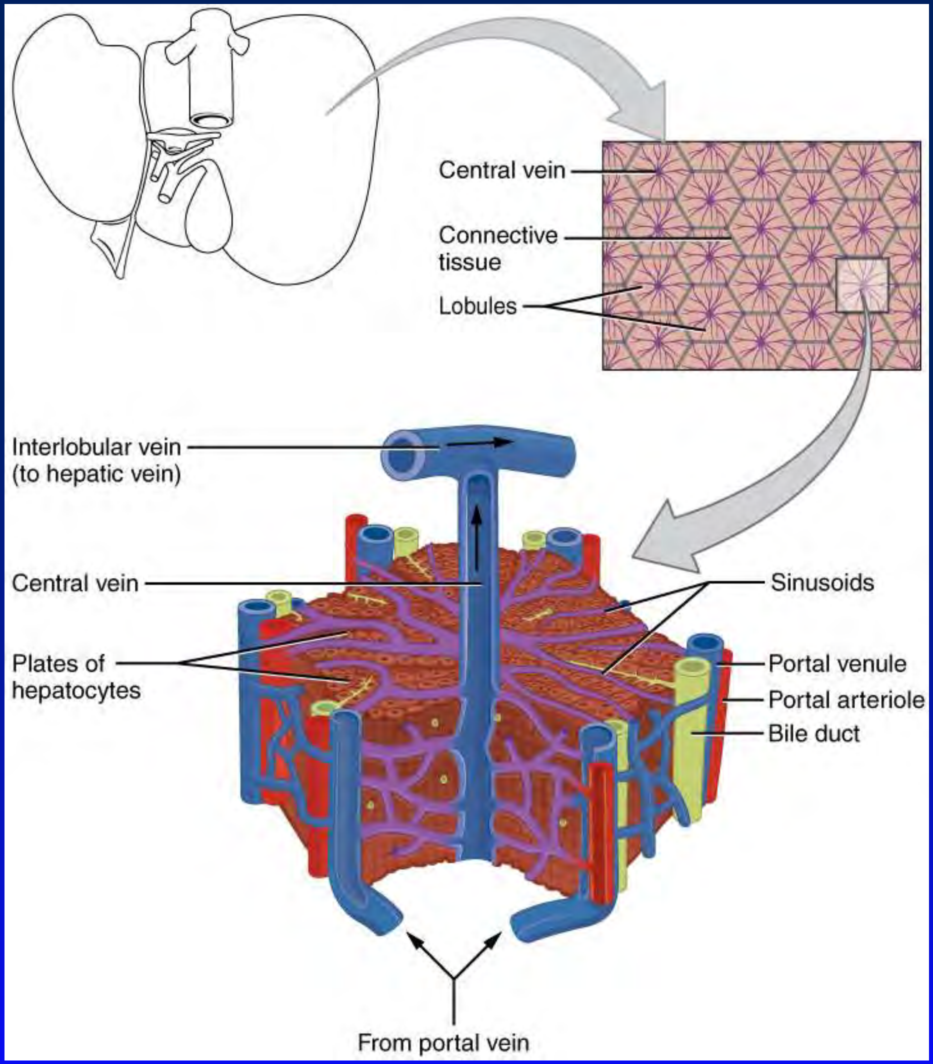
Liver Failure

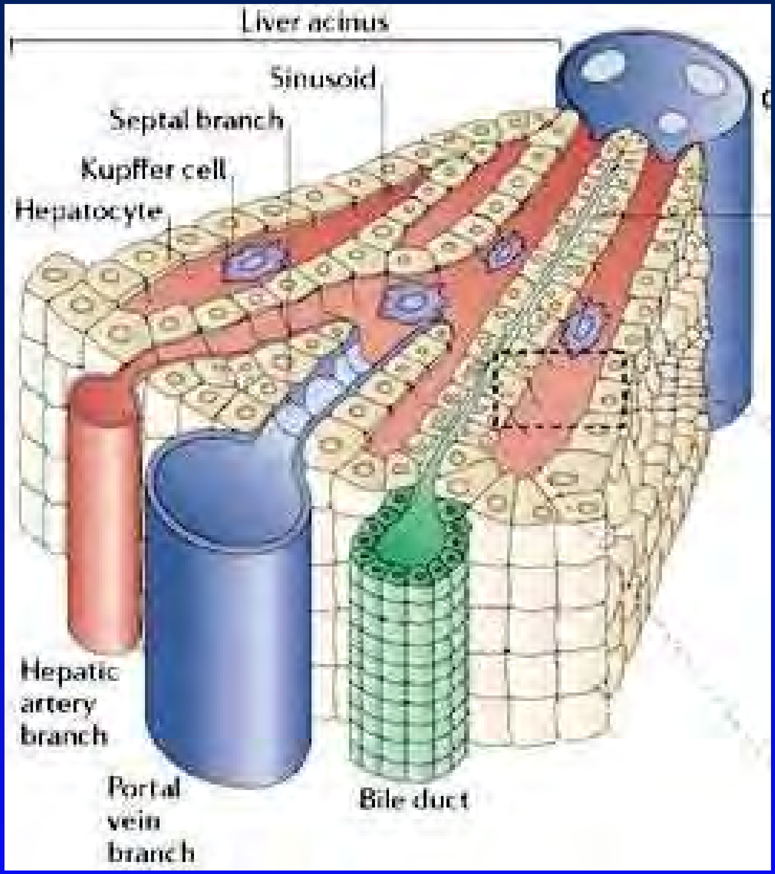
- Encephalopathy
- Fluid shifts (ascites)
- Coagulopathy
- Jaundice
- Immunosuppressed

Liver Gross Anatomy



Liver Microanatomy





Cell Types That Can Regenerate Liver

- Mature Hepatocytes
  - Major mechanism for regeneration
  - Serial hepatocyte transplantation show mature hepatocytes can divide >70 times
- Stem Cells
  - Liver Tissue-based Stem Cells
  - Embryonic Stem Cells
  - Induced Pluripotent Stem Cells (iPS Cells)

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"Off-the-Shelf" Availability
HLA-matched iPSC Banks

3 loci match at HLA-A, B, DR:

- Japan – 50 lines to match 90% of population
  (Nakatsuji et al., *Nat Biotech*, 2008)
- UK – 150 lines to match 90% of population
  (Taylor et al., *Cell Stem Cell*, 2012)
- North America – 900 lines to match 95% of population
  (https://www.the-scientist.com/?articles.view/articleNo/40376/title/Banking-on-iPSCs/)

Agenda

- Why tissue engineering
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Liver Tissue Engineering – 3 Major Approaches

- Prescribed design
- Decellularized scaffold
- Self-assembly

Prescribed Design

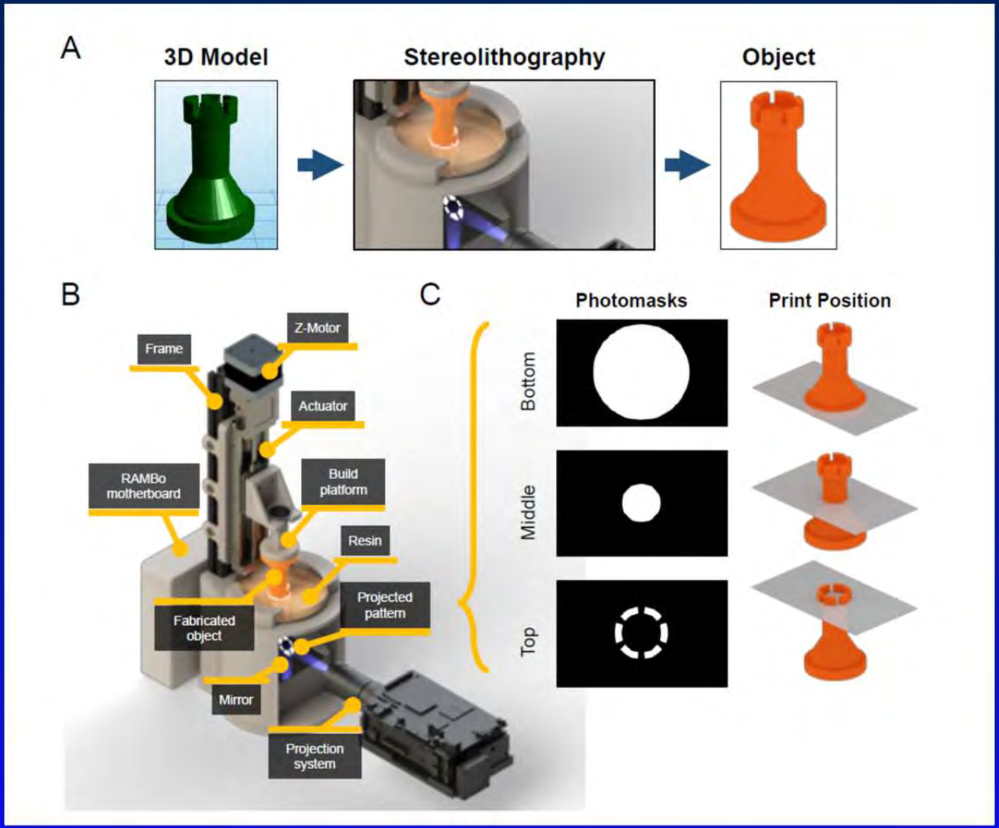
Multivascular networks and functional intravascular topologies within biocompatible hydrogels

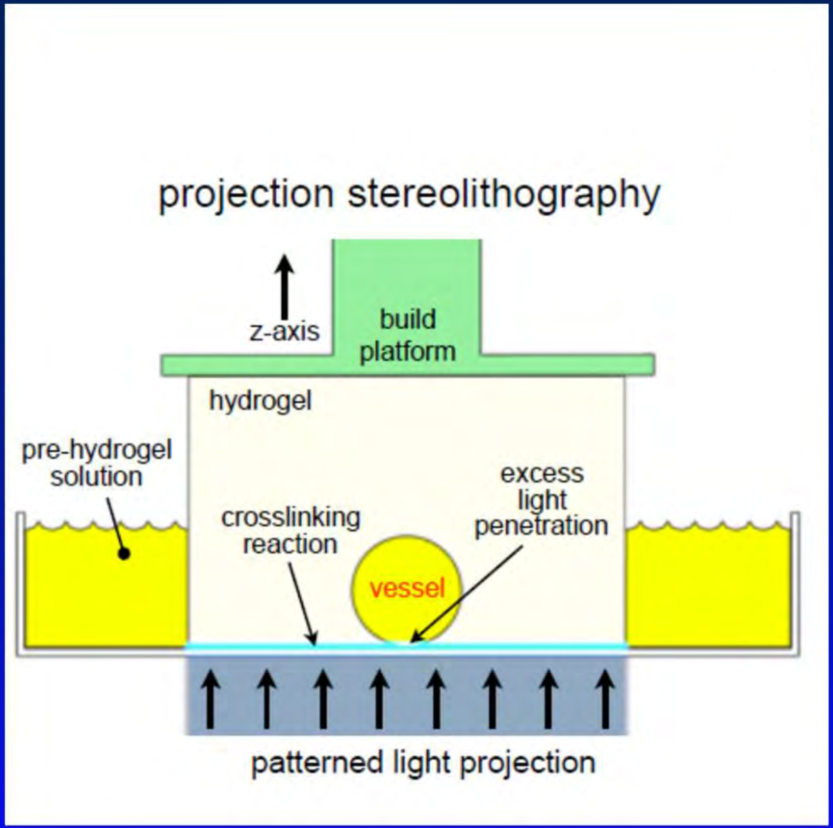
Bagrat Grigoryan[1]*, Samantha J. Paulsen[1]*, Daniel C. Corbett[2,3]*, Daniel W. Sazer[1], Chelsea L. Fortin[3,4], Alexander J. Zaita[1], Paul T. Greenfield[1], Nicholas J. Calafat[1], John P. Gounley[5]†, Anderson H. Ta[1], Fredrik Johansson[2,3], Amanda Randles[5], Jessica E. Rosenkrantz[6], Jesse D. Louis-Rosenberg[6], Peter A. Galie[7], Kelly R. Stevens[2,3,4]‡, Jordan S. Miller[1]‡

Grigoryan *et al.*, *Science* **364**, 458–464 (2019)   3 May 2019

Grigoryan et al., Science, 2019

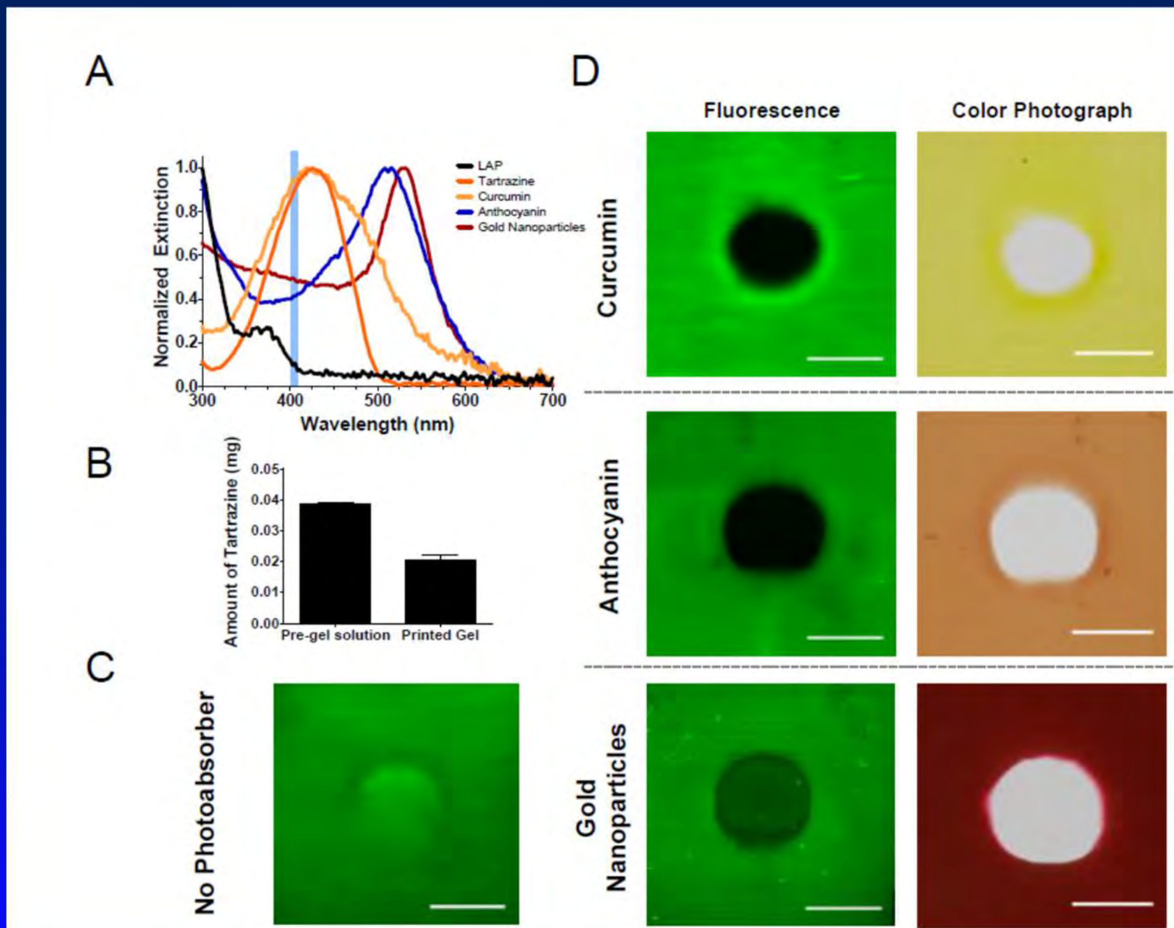
Projection Photolithography





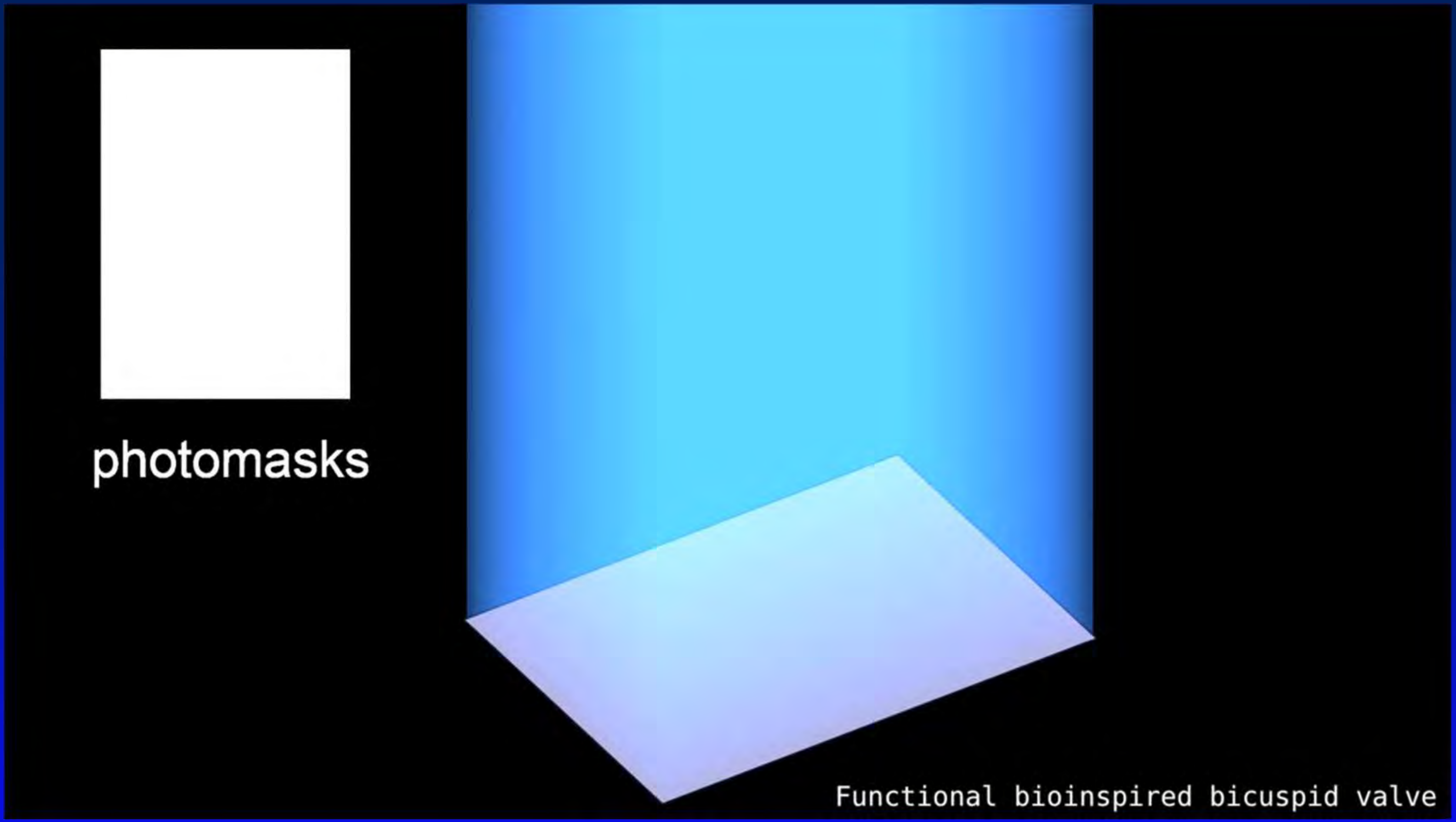
Grigoryan et al., Science, 2019

Photo Absorber – Tartrazine (Yellow Food Coloring)



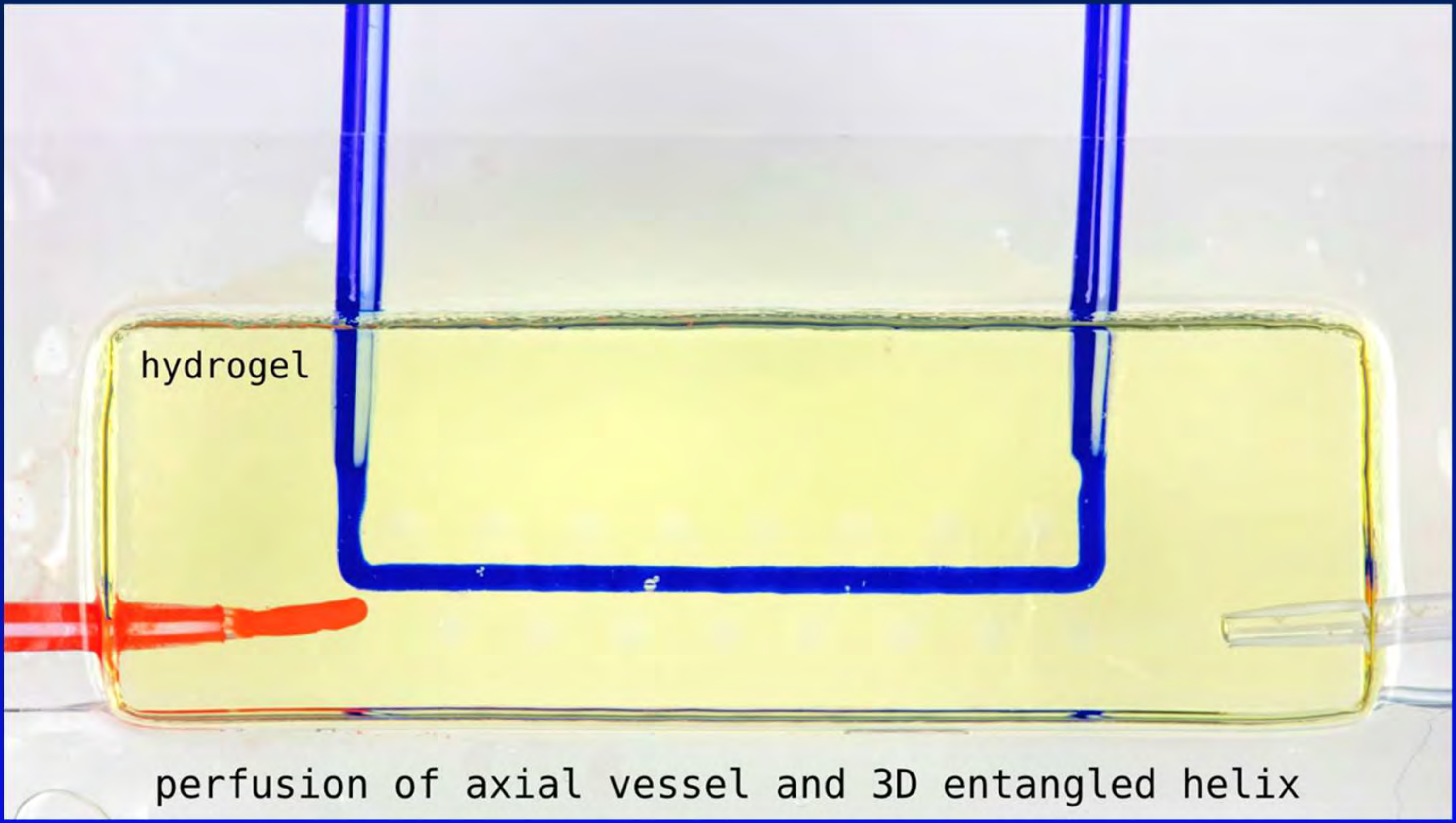
Grigoryan et al., Science, 2019

Print Vessels with Valves



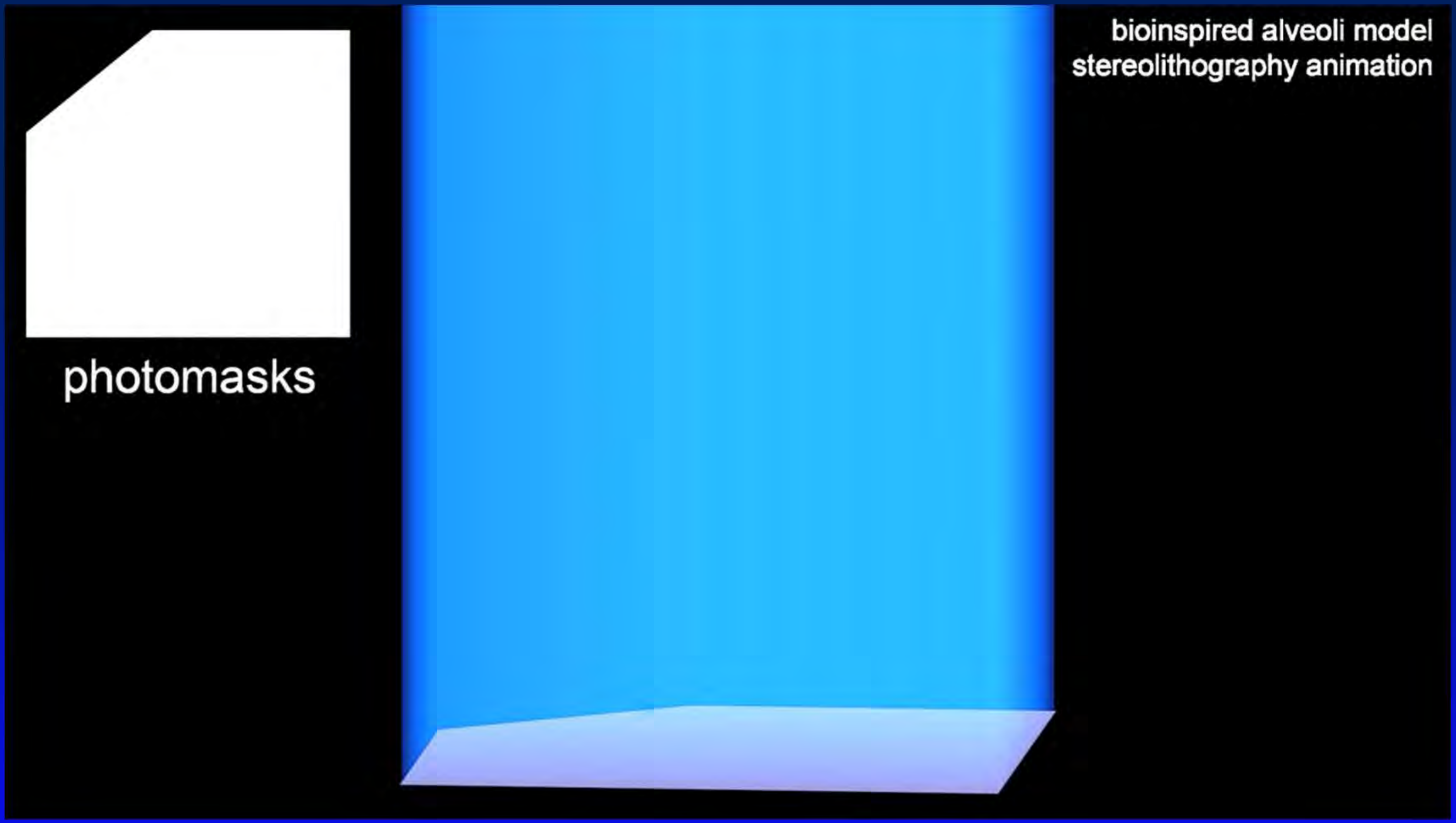
(Grigoryan et al., Science, 2019)

Print Complex Intertwined Vasculature



Grigoryan et al., Science, 2019

Print Lung Alveolus



Grigoryan et al., Science, 2019

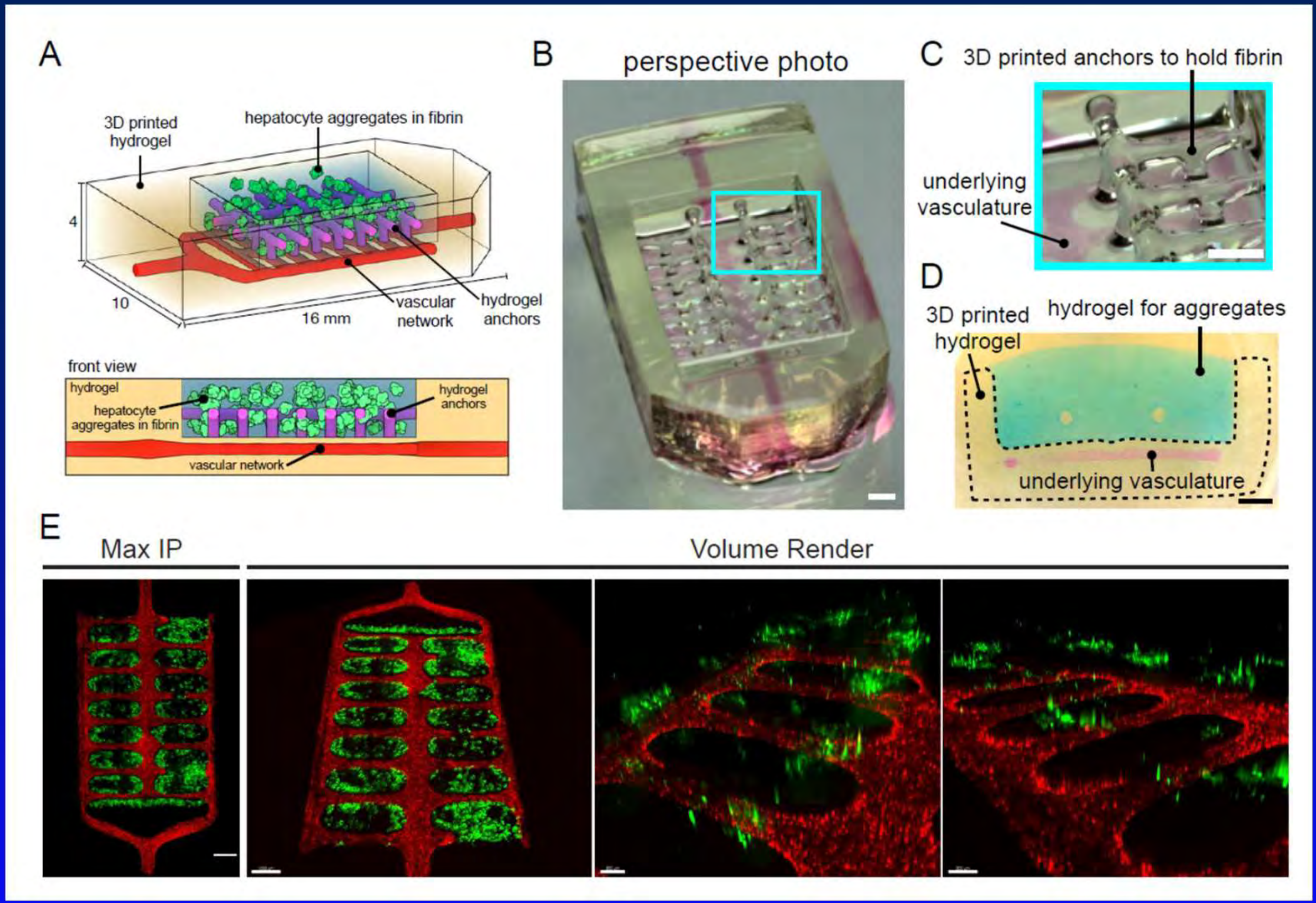
Print Lung

Generative, scalable
lung-mimetic design

Step 1: Grow airway within the bounding volume

Grigoryan et al., Science, 2019

Liver Implant



Grigoryan et al., Science, 2019

Prescribed Design

Pros:

- Control over design

Challenges:

- Design may not be biologically complex enough
- Biocompatibility of hydrogel

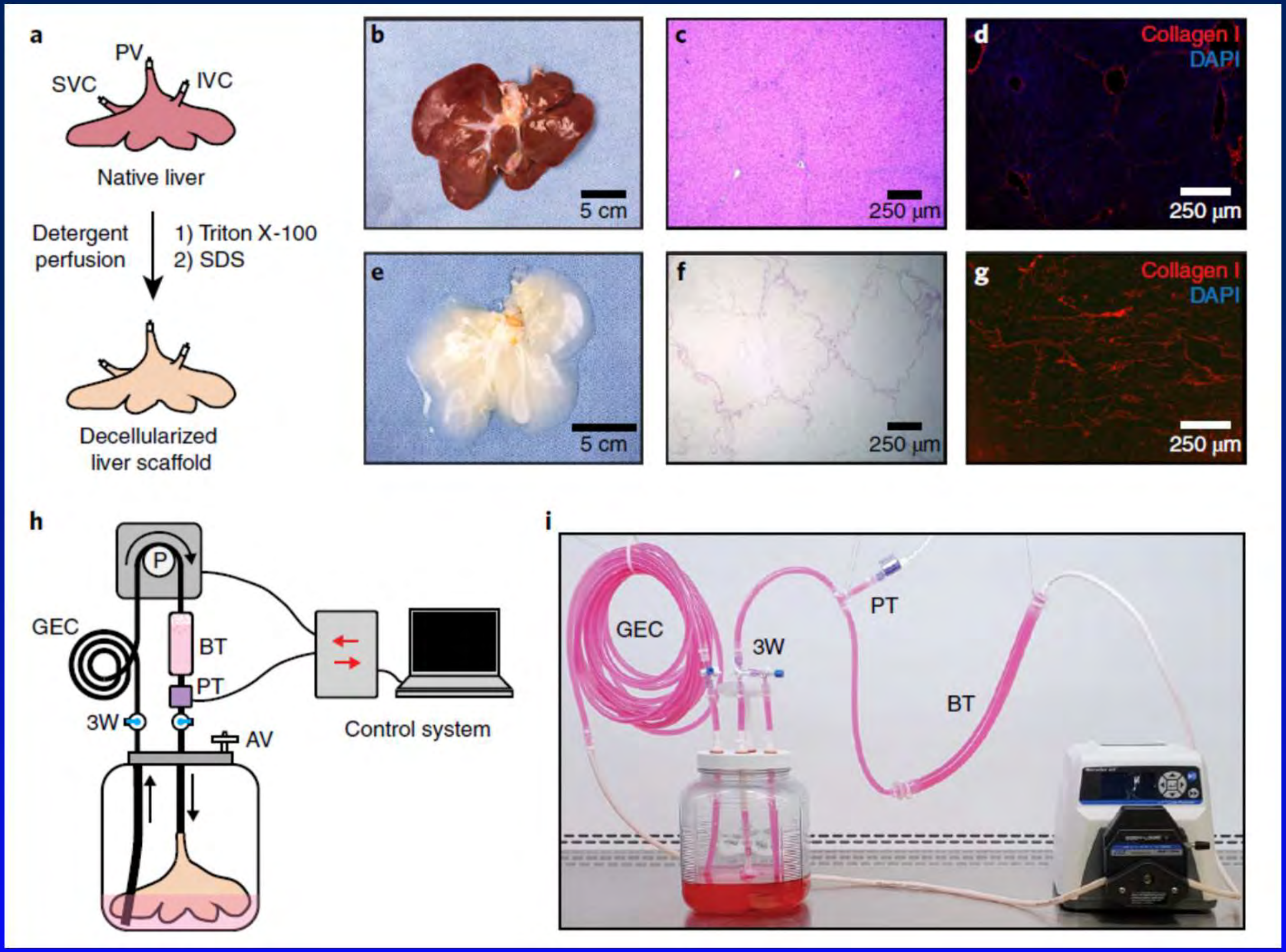
Decellularized Scaffold

Sustained perfusion of revascularized bioengineered livers heterotopically transplanted into immunosuppressed pigs

Mohammed F. Shaheen[1,2,11], Dong Jin Joo[1,3,11], Jeffrey J. Ross[4*], Brett D. Anderson[4], Harvey S. Chen[1,2], Robert C. Huebert[5], Yi Li[1], Bruce Amiot[1], Anne Young[4], Viviana Zlochiver[4], Erek Nelson[1,2], Taofic Mounajjed[6], Allan B. Dietz[6], Gregory Michalak[7], Benjamin G. Steiner[4], Dominique Seetapun Davidow[4], Christopher R. Paradise[8], Andre J. van Wijnen[9,10], Vijay H. Shah[5], Mengfei Liu[5] and Scott L. Nyberg[1,2*]

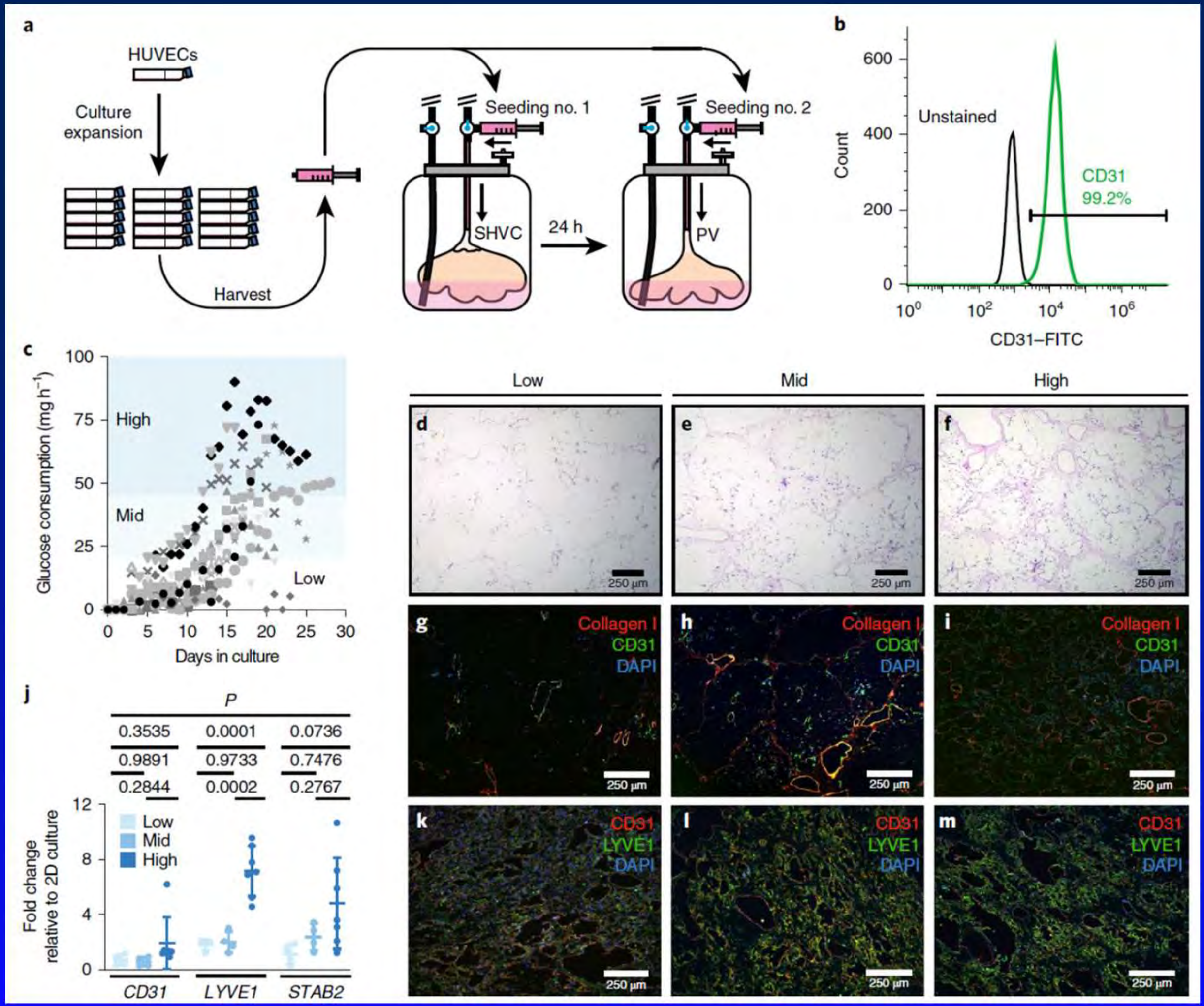
NATURE BIOMEDICAL ENGINEERING | VOL 4 | APRIL 2020 | 437–445 |

Decellularization



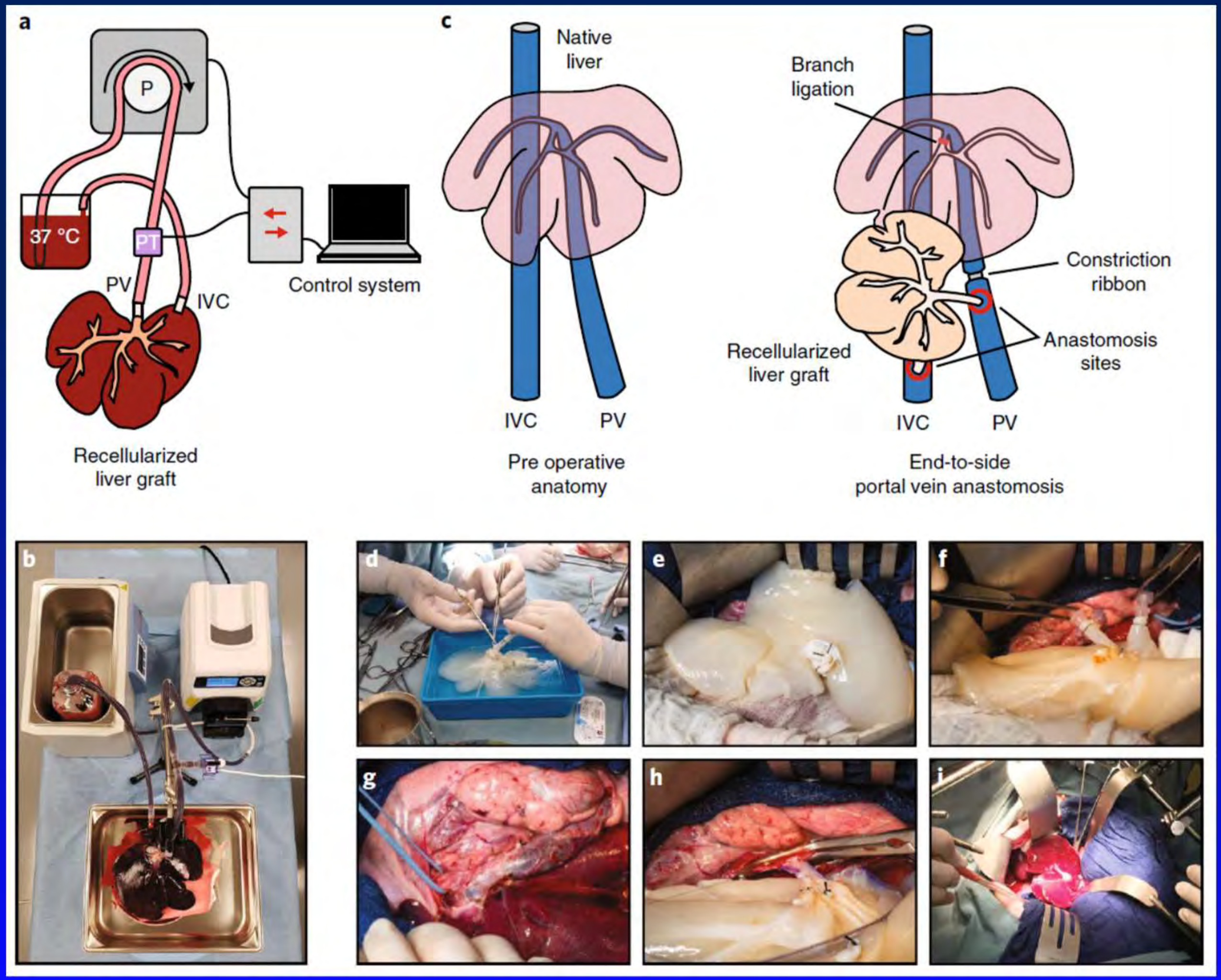
Shaheen et al., Nat Biomed Eng, 2020

Recellularization



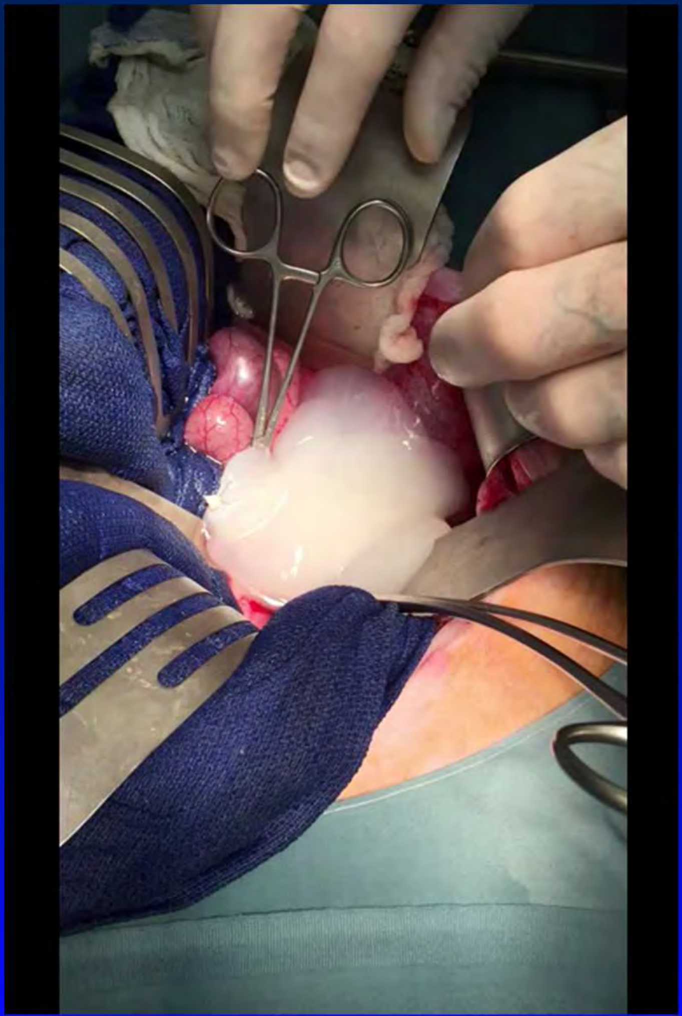
Shaheen et al., Nat Biomed Eng, 2020

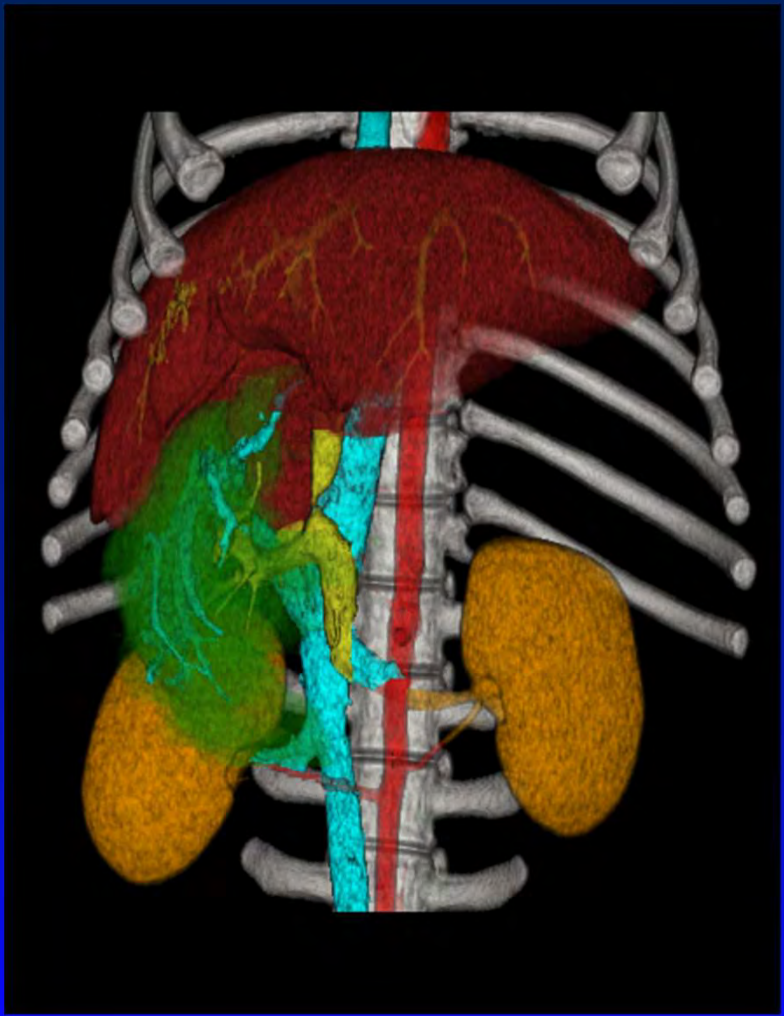
Implantation



Shaheen et al., Nat Biomed Eng, 2020

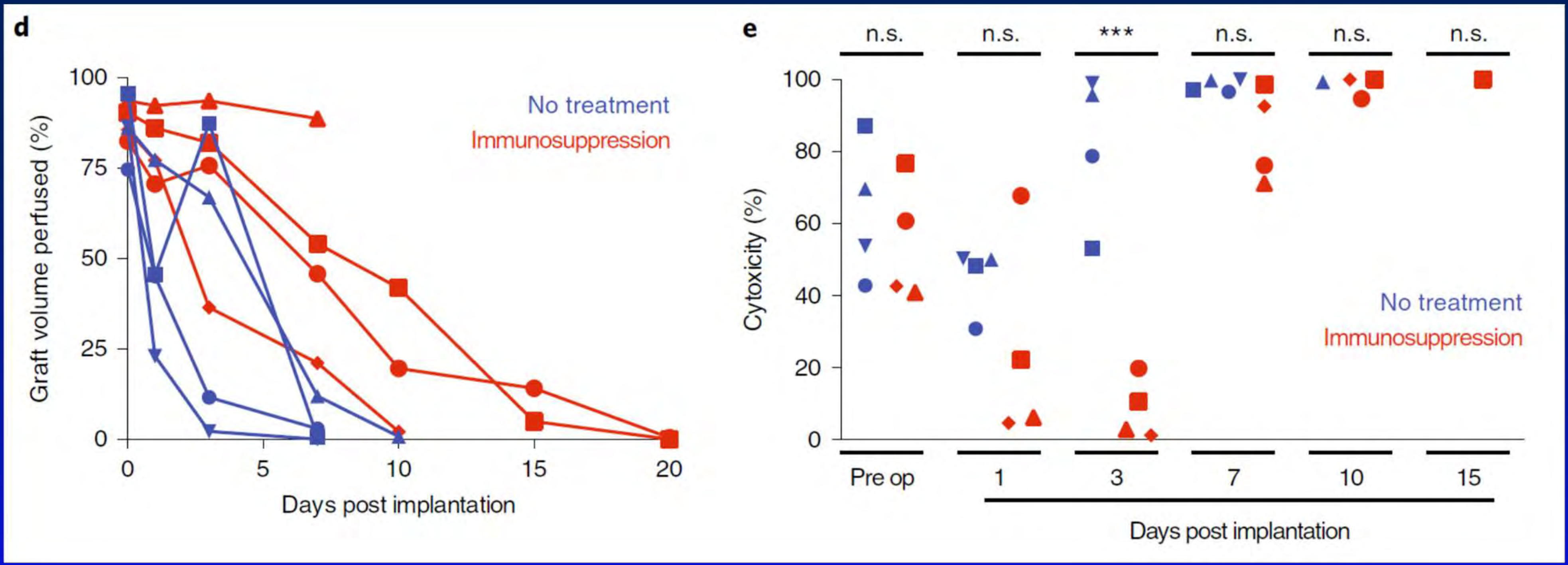
Implantation





Shaheen et al.,
Nat Biomed Eng,
2020

Graft Viability Limited



Shaheen et al., Nat Biomed Eng, 2020

Decellularized Scaffold

Pros:

- Native matrix
- Innate complex branching and micro-architecture

Challenges:

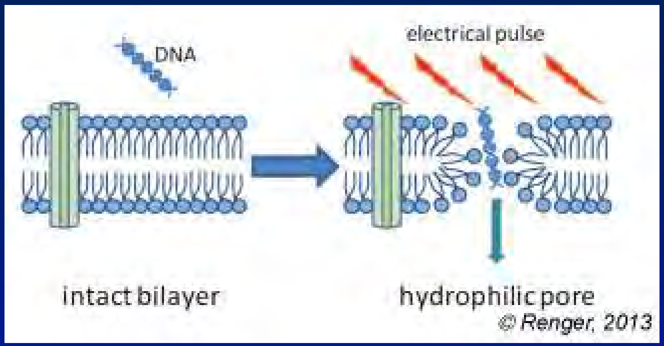
- Requires biological source (human or animal)
- Cross-species immune and infectious barriers
- Efficiency of recellularization
- Thrombogenesis

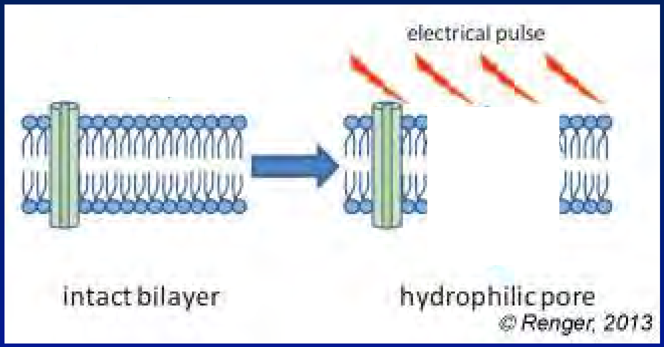
Irreversible Electroporation (IRE)

- Marketed as Nanoknife
- Non-thermal ablation
- Causes cell death with minimal inflammation
- Leaves extracellular matrix scaffold intact
- Induces scarless healing

Davalos, Mir, and Rubinsky, 2005

Reversible versus Irreversible Electroporation

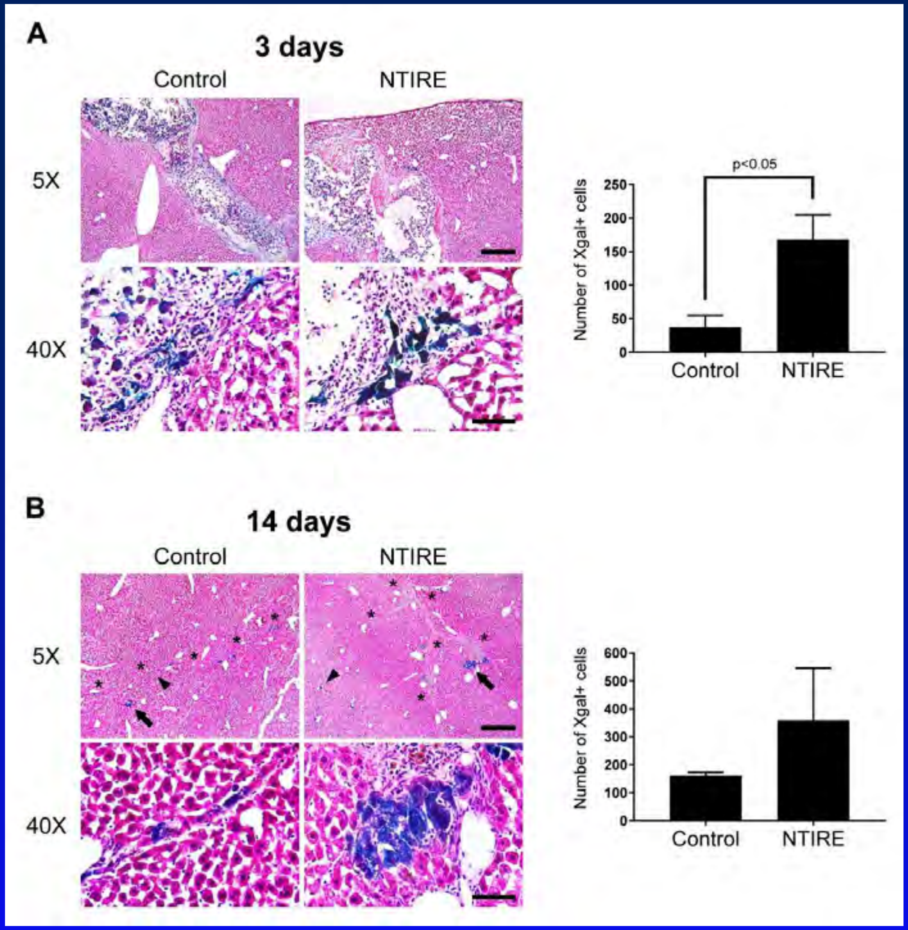




Gene Expression
Electrochemotherapy

Cell Death

Irreversible Electroporation as a Tool for In Vivo Decellularization



Chang TT, Zhou, and Rubinsky, Biotechniques, 2017

Self-Assembly

Modelling human hepato–biliary–pancreatic organogenesis from the foregut–midgut boundary

Hiroyuki Koike[1,2], Kentaro Iwasawa[1,2], Rie Ouchi[1,2], Mari Maezawa[1,2], Kirsten Giesbrecht[1,2], Norikazu Saiki[3], Autumn Ferguson[1,2], Masaki Kimura[1,2], Wendy L. Thompson[1,2], James M. Wells[2,4,5,6], Aaron M. Zorn[2,4,6] & Takanori Takebe[1,2,3,4,6]*

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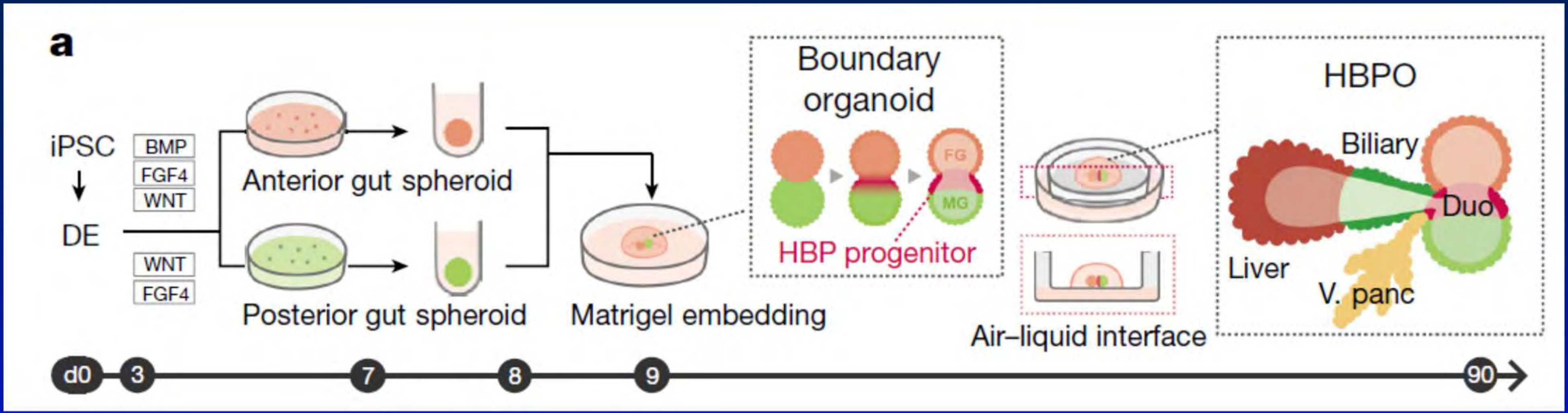
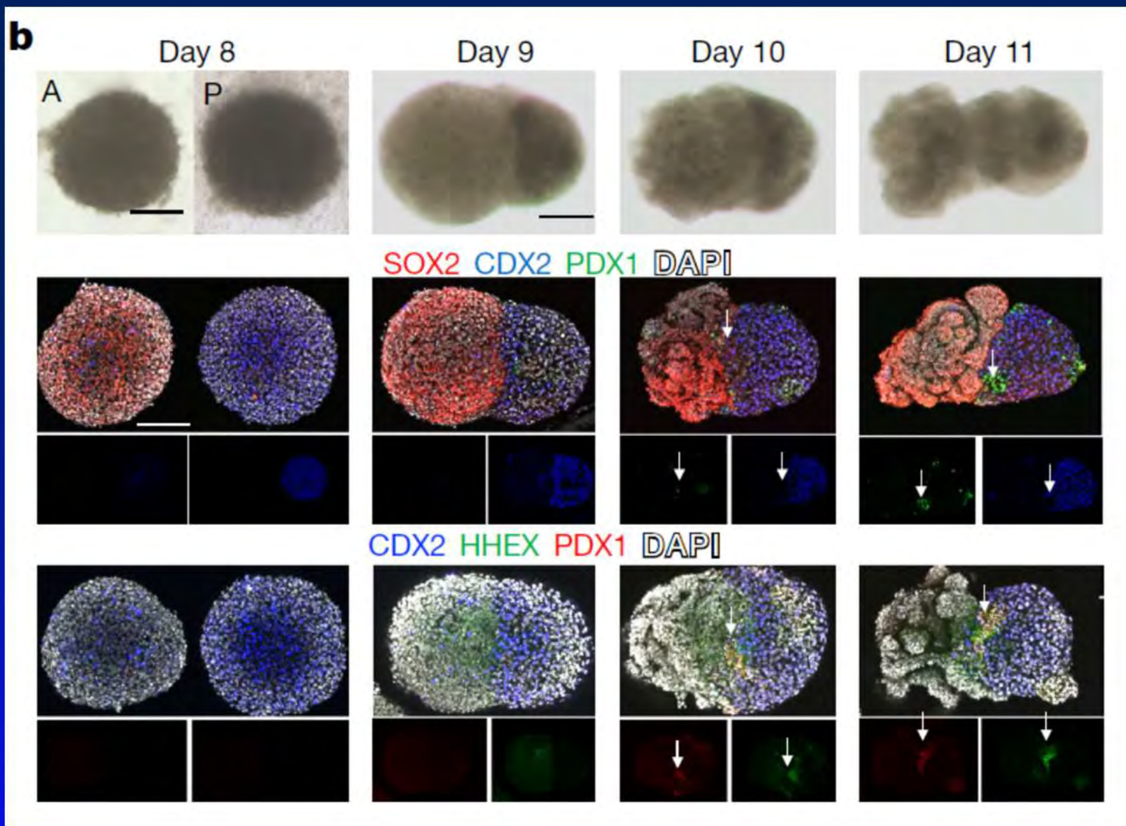
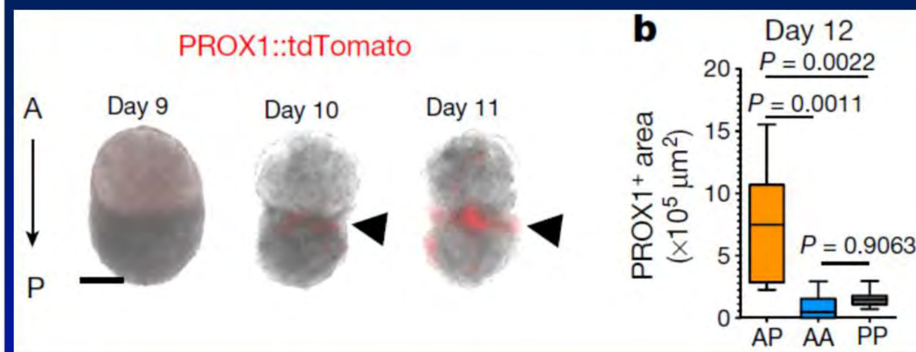
Organoid Cell Fate Specification without Exogenous Factors



Koike et al., Nature, 2019

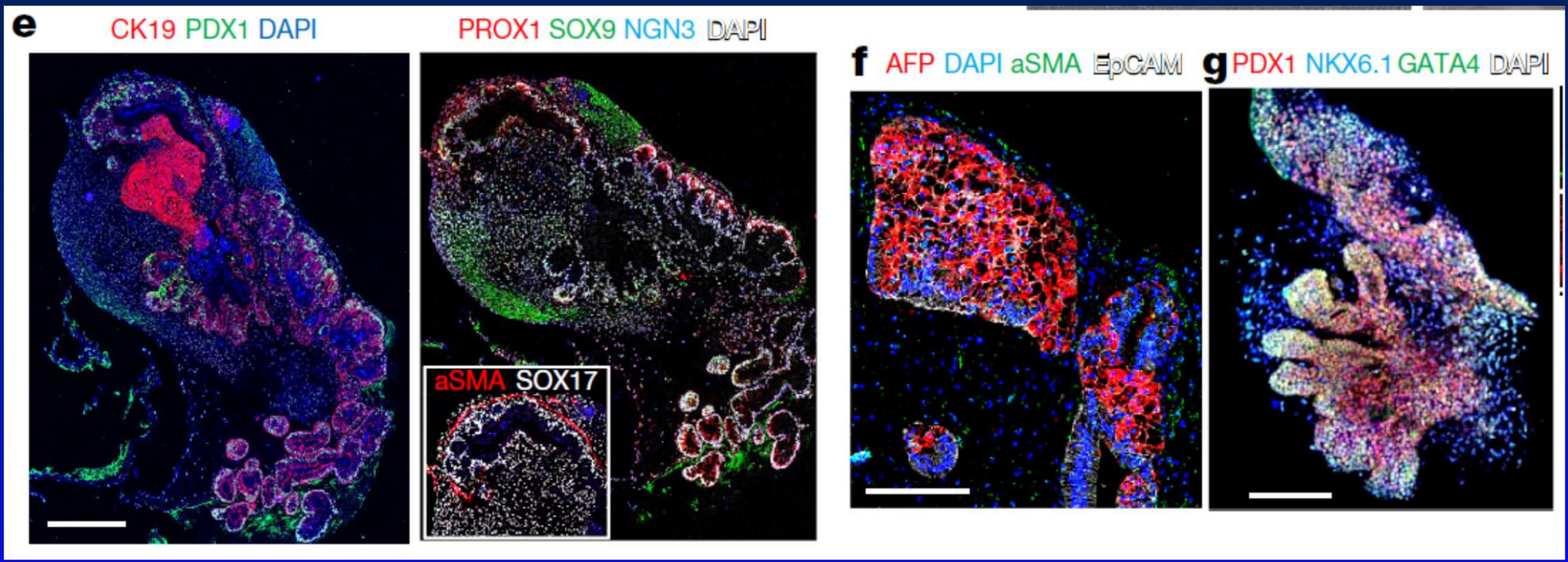
Inductive Signals at Organoid Fusion Interface





Koike et al., Nature, 2019

Liver, Biliary, and Pancreatic Lineages with Tissue Organization



Koike et al., Nature, 2019

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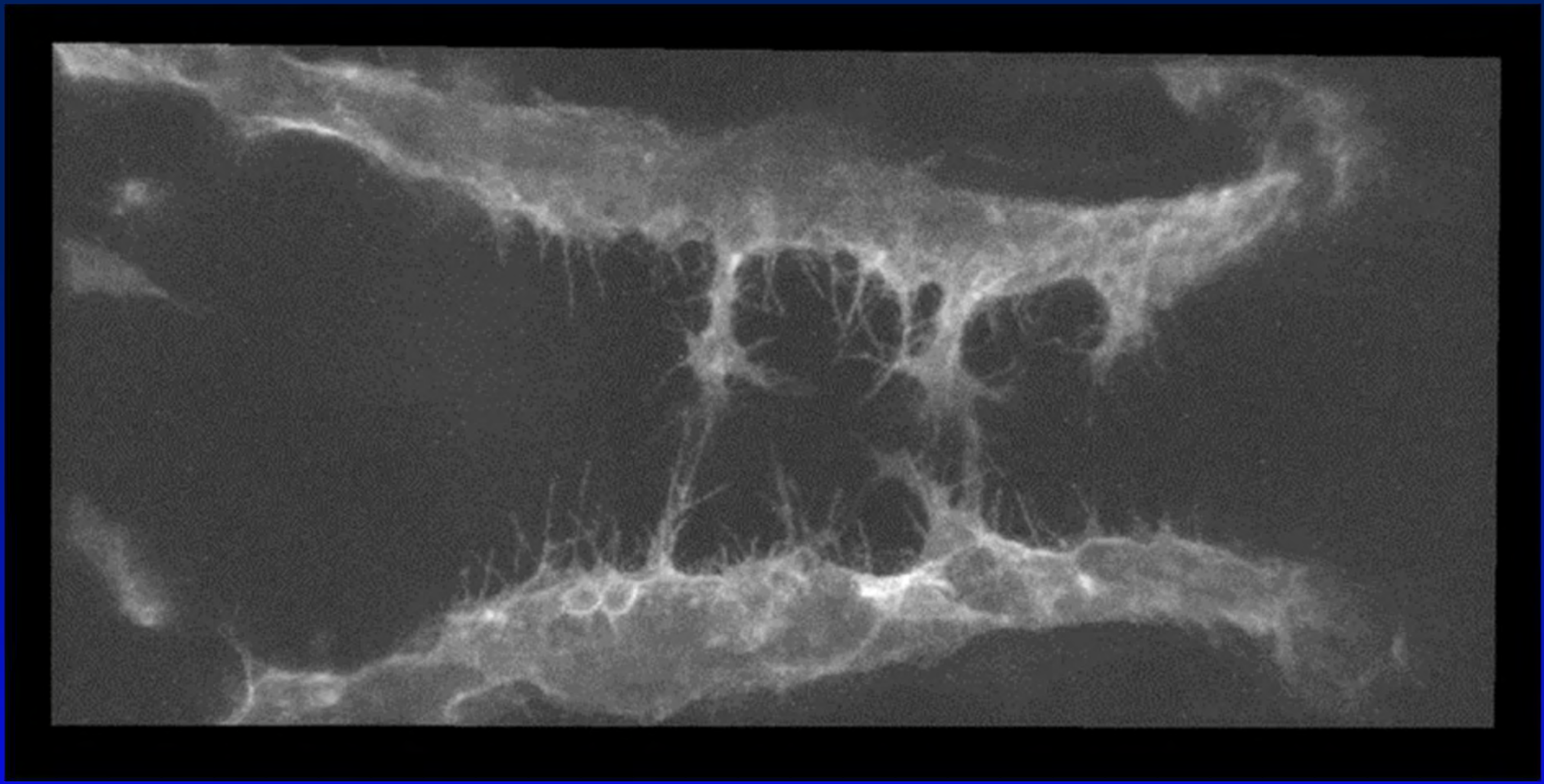
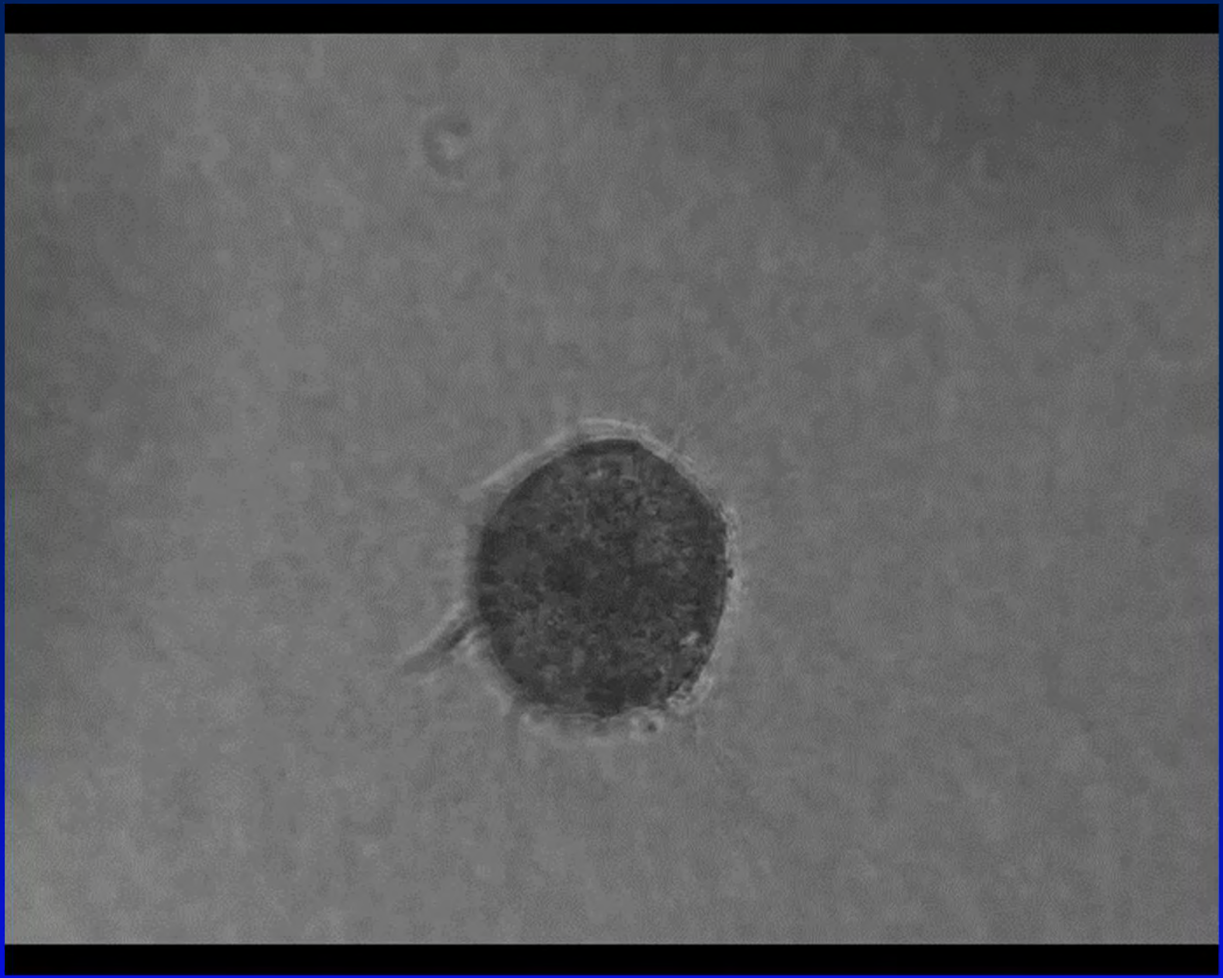
Development as Tissue Engineering's Teacher



Bussmann JS et al., Development, 2011



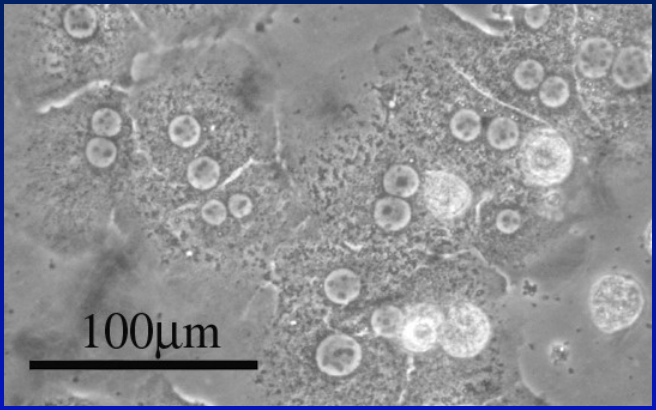
Heiss M et al., *FASEB J*, 2015

Rotating Wall Vessel Bioreactors

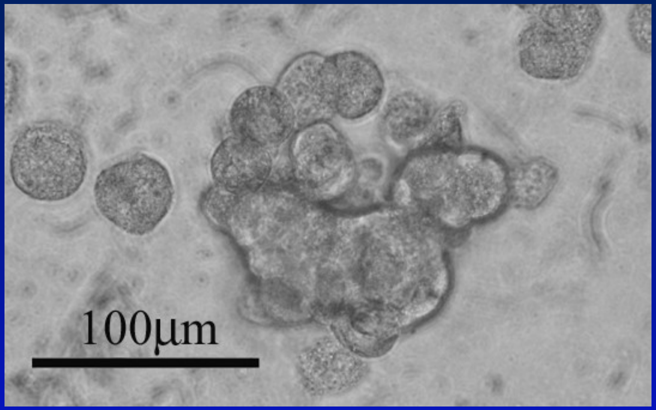
- Low shear stress
- Low turbulence
- Three-dimensional environment
- Allow self-association and self-organization
- Simulated microgravity

Monolayer vs. Organoid Culture

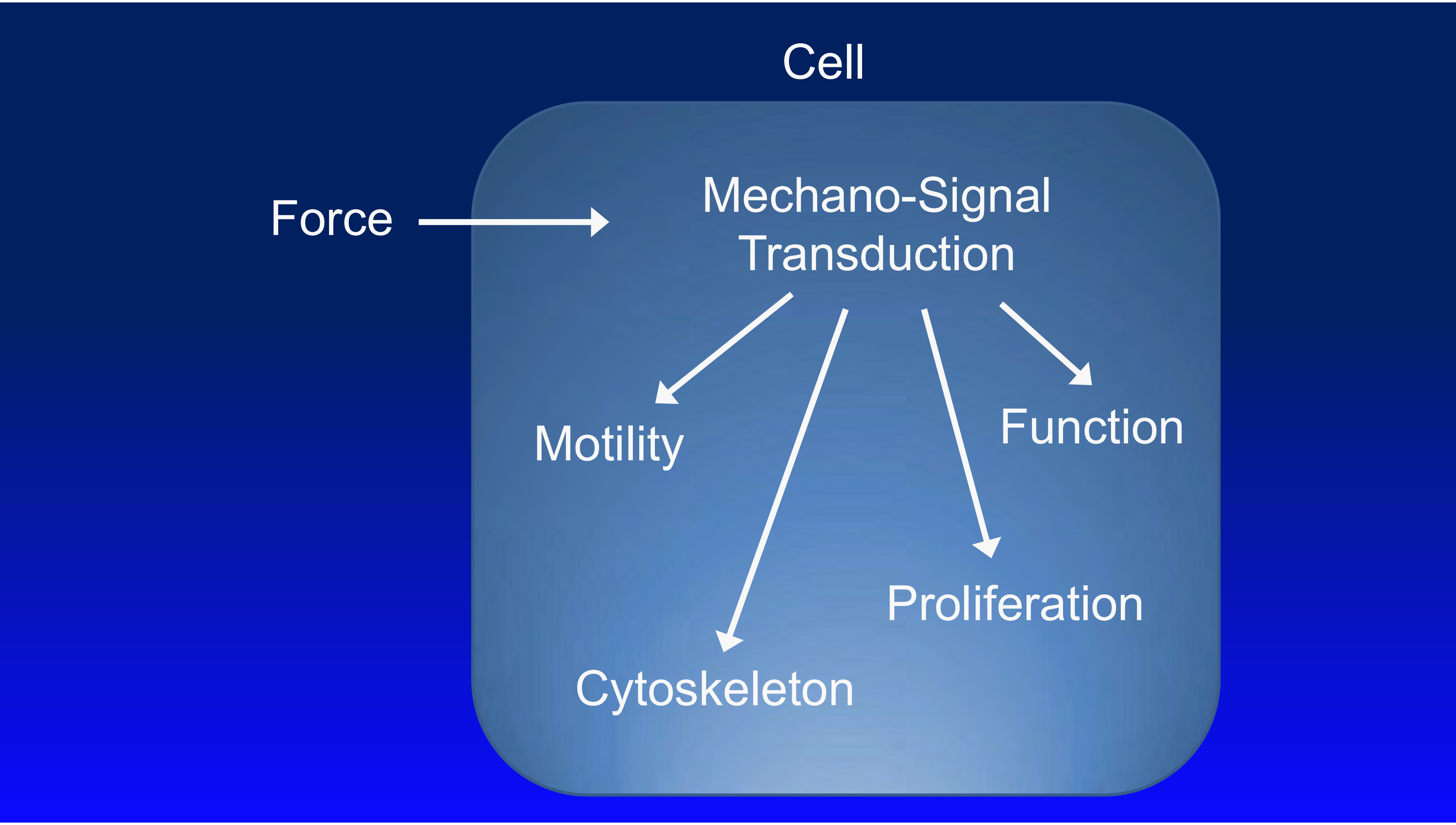
Hepatocytes



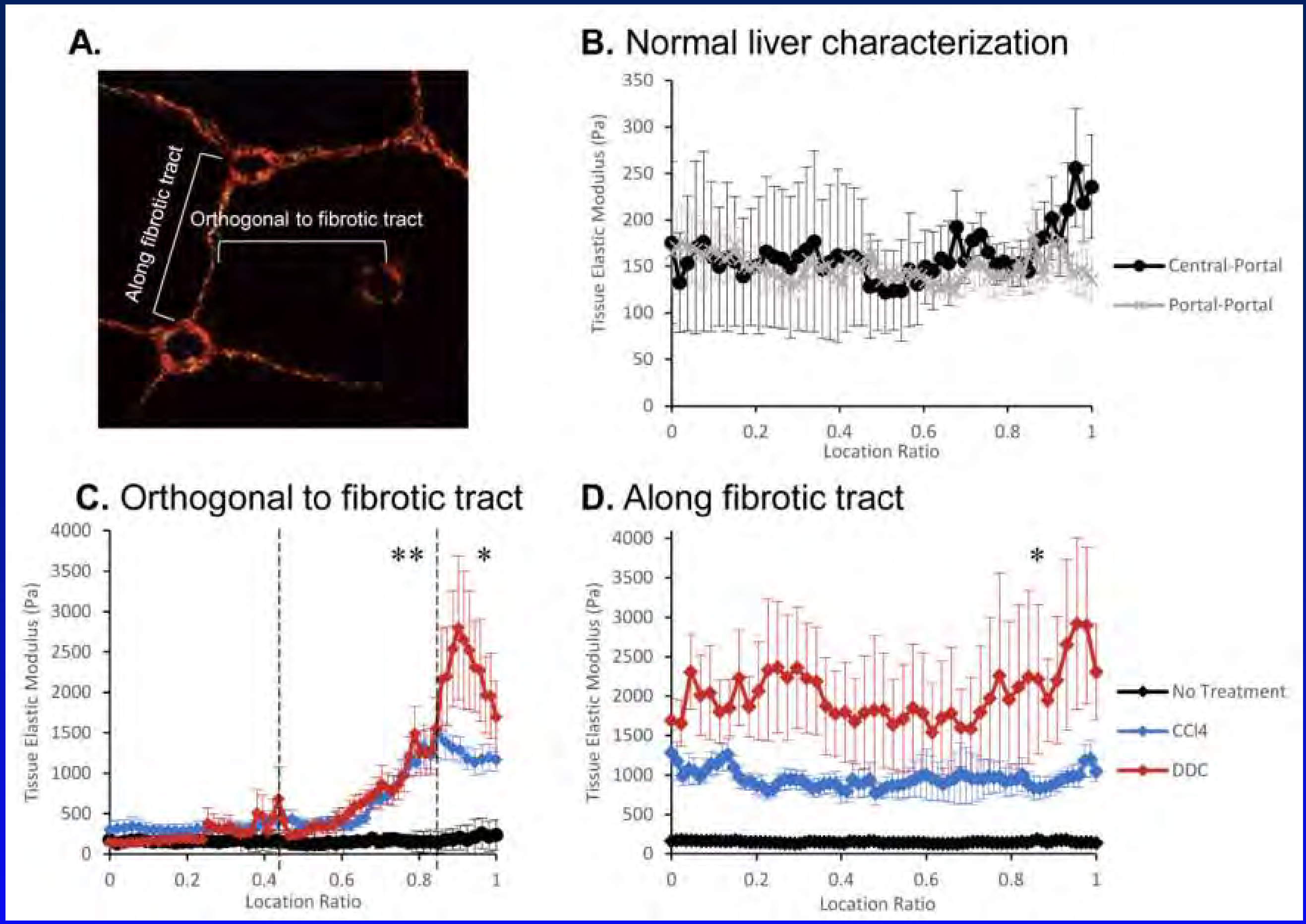
Tissue Culture Dish



Rotating Wall Vessel

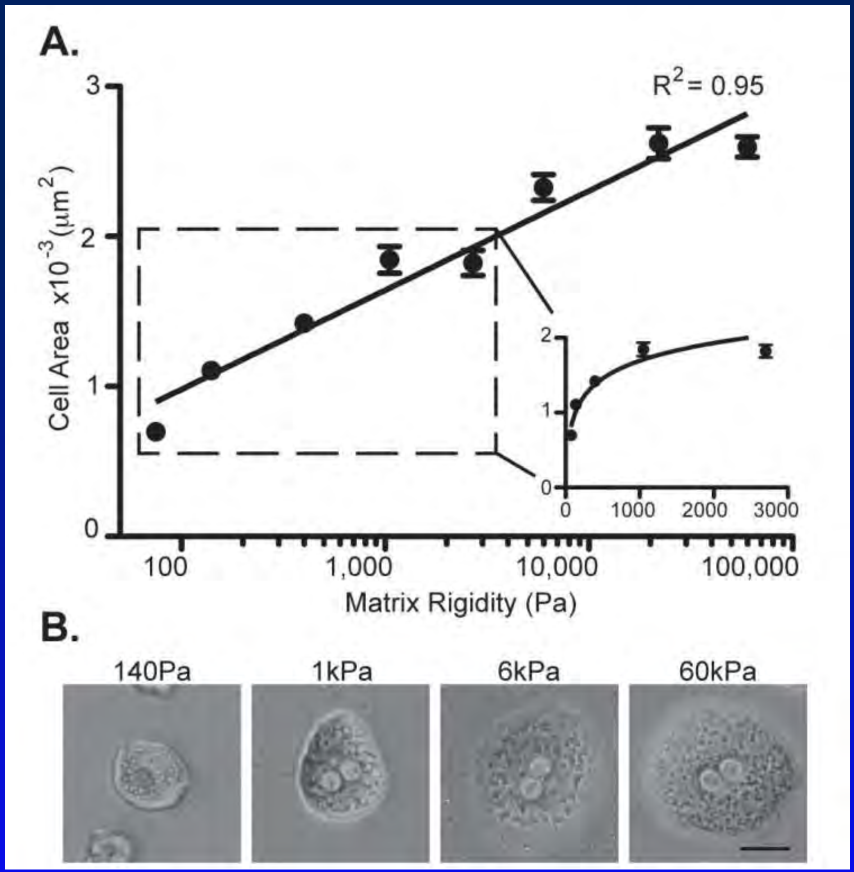

Cell
Force
Mechano-Signal
Transduction
Motility
Function
Proliferation
Cytoskeleton

Liver fibrosis results in region specific increases in tissue matrix stiffness



Desai and Tung et al., Hepatology, 2016

Force Affects Cell Spreading

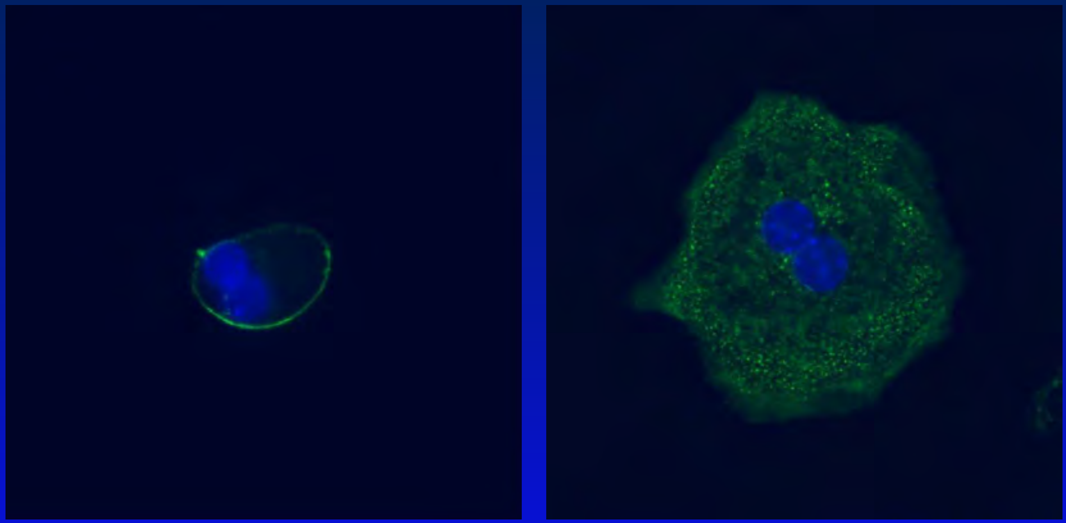


Desai and Tung et al., Hepatology, 2016

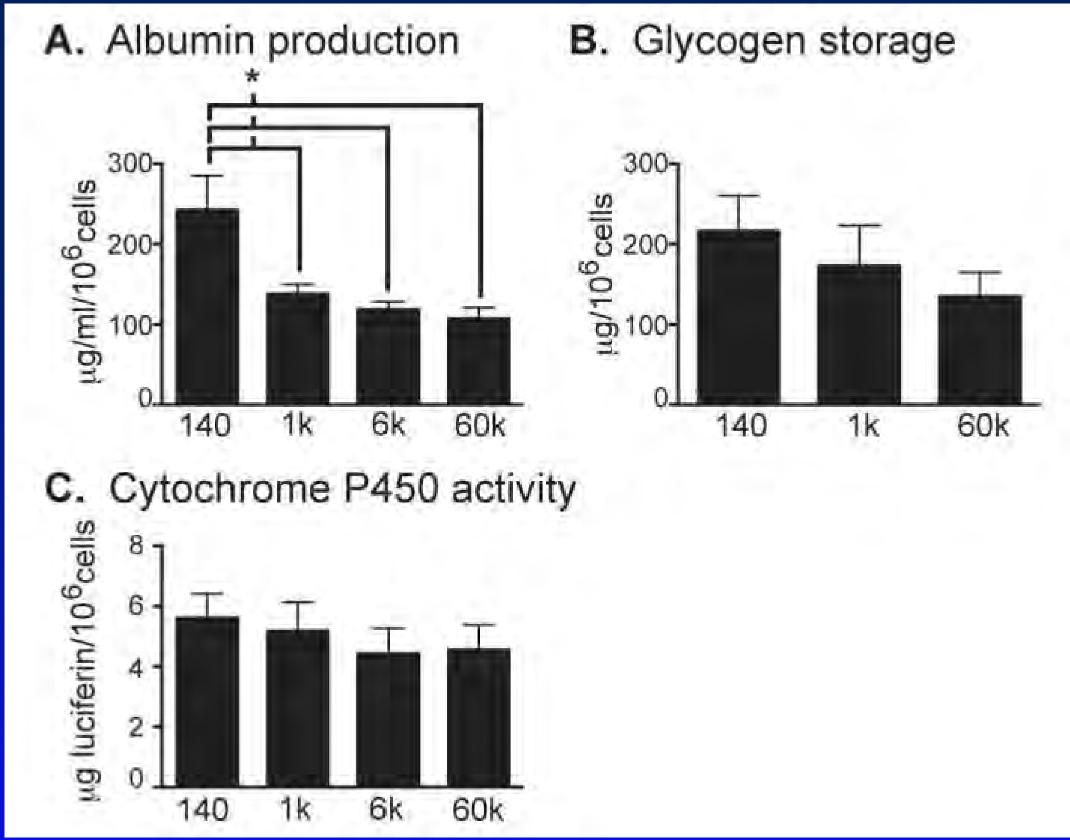
Force Affects Cytoskeletal Organization

140 Pa

1kPa

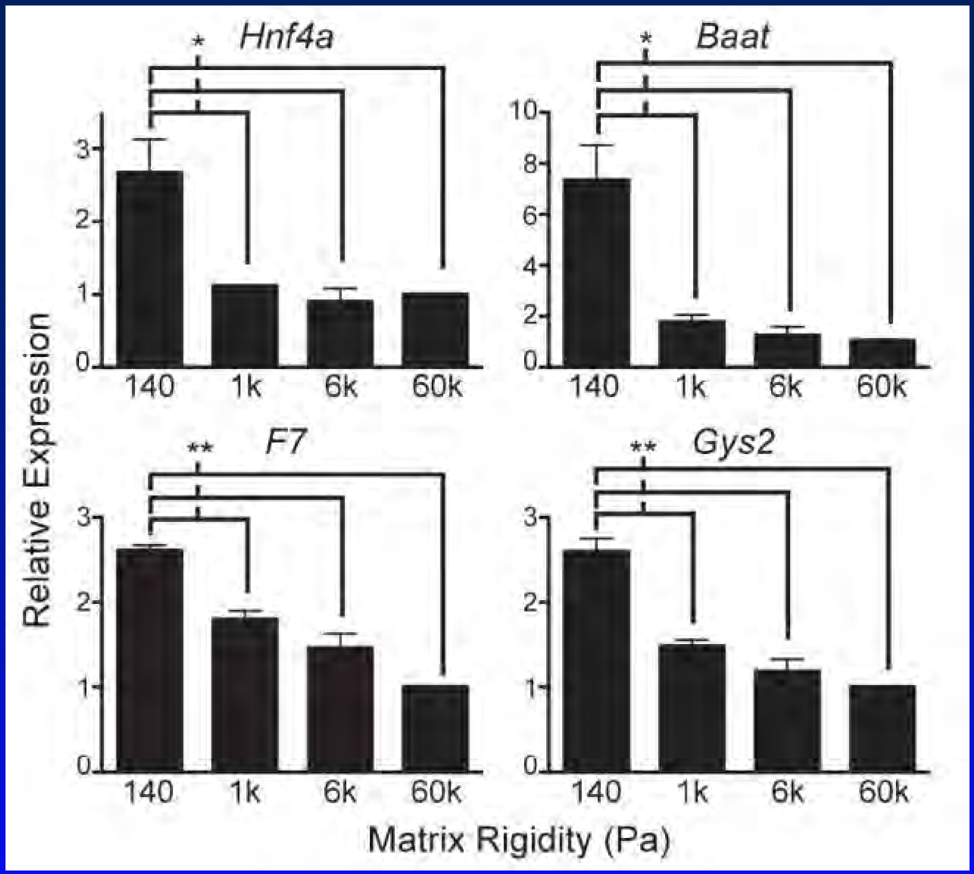


Force Affects Function



Desai and Tung et al., Hepatology, 2016

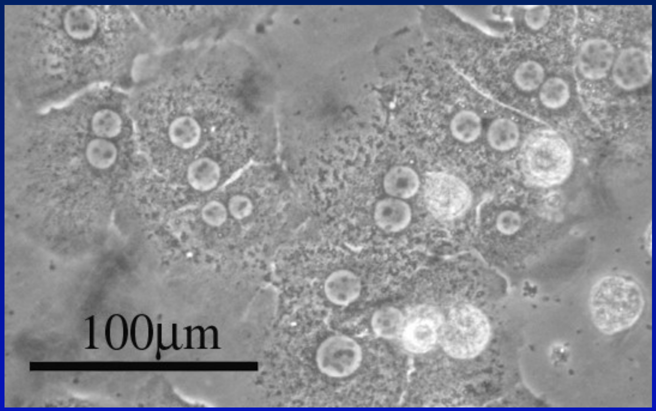
Force Affects Gene Expression



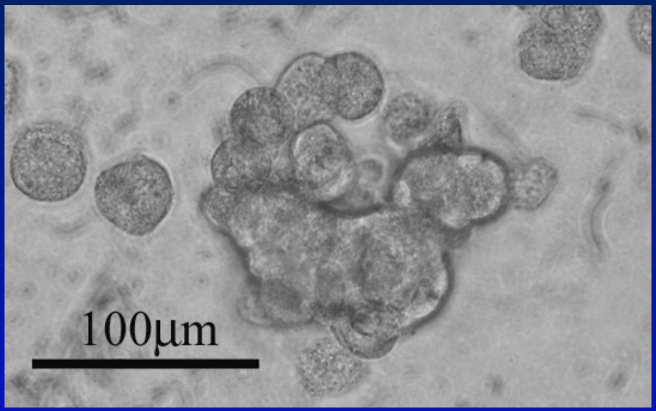
Desai and Tung et al., Hepatology, 2016

Monolayer vs. Organoid Culture

Hepatocytes

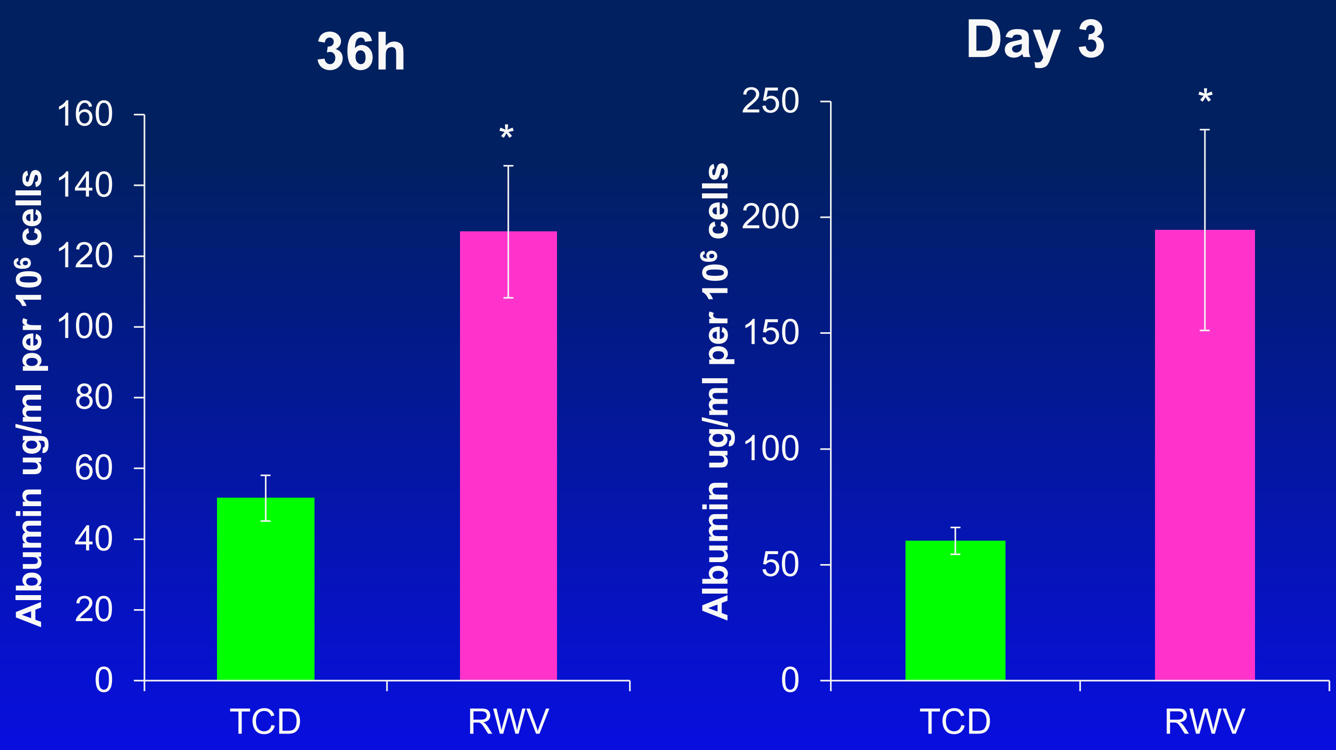


Tissue Culture Dish



Rotating Wall Vessel

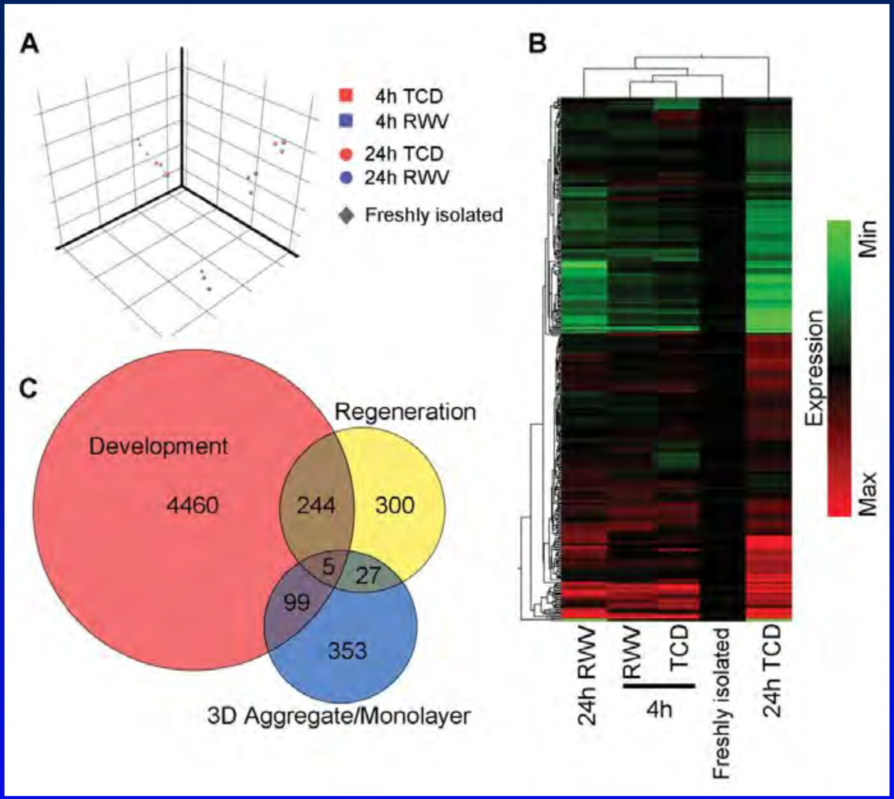
Albumin Production is Greater in RWV Organoid Cultures



*p<0.05

Chang TT and Hughes-Fulford M, Biomaterials, 2014

Upregulated Genes in Hepatic Organoids are Distinct from those Upregulated in Liver Development and Regeneration



Chang TT and Hughes-Fulford M, Biomaterials, 2014

Biological Processes Upregulated in Hepatic Organoids

GO term	Number of genes	P-value
Lipid metabolic process	44	<0.0001
Organic acid metabolic process	41	<0.0001
Amino acid metabolic process	19	<0.01
Organic acid biosynthetic process	23	<0.0001
Amino acid biosynthetic process	12	<0.001
Oxidation-reduction process	35	<0.05
Response to glucose stimulus	9	<0.05
Response to xenobiotic stimulus	6	<0.05

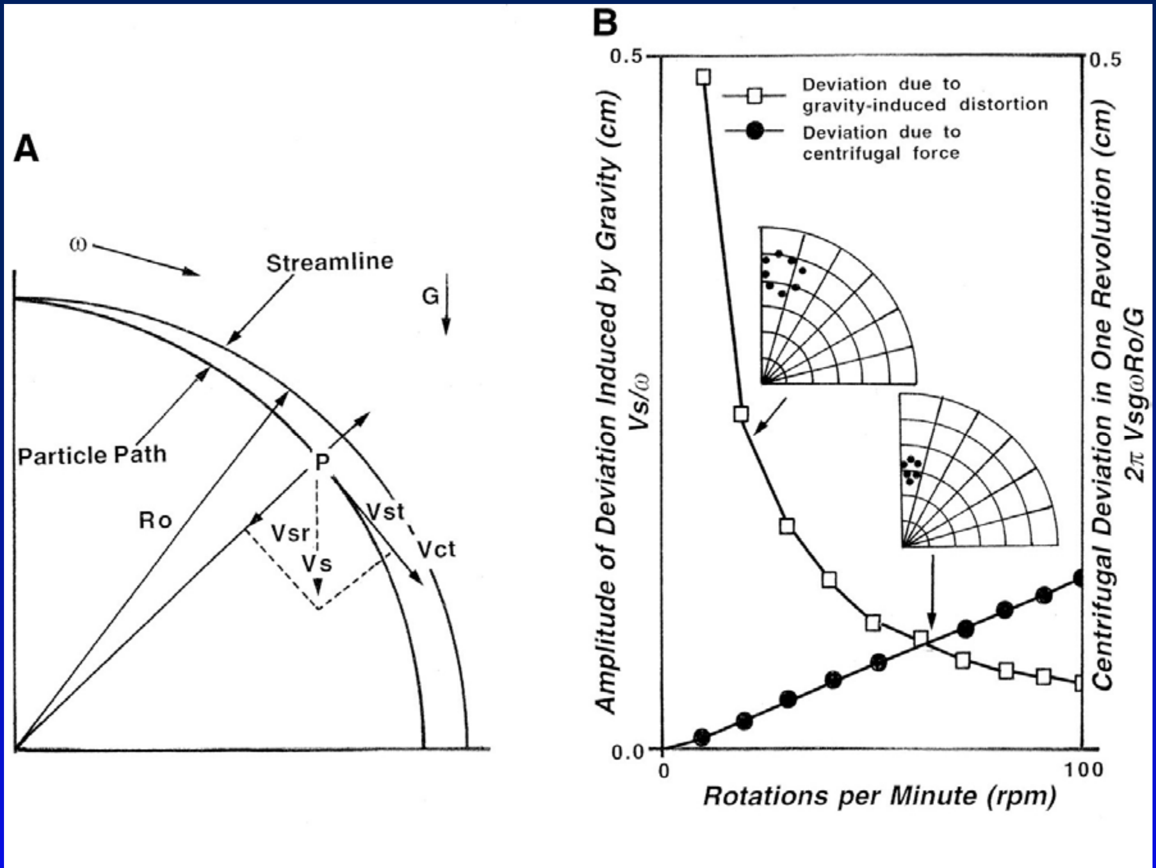
Chang TT and Hughes-Fulford M, Biomaterials, 2014

HNF4a Binding Sites are Significantly Over-Represented in Genes Upregulated in Hepatic Organoids

Transcription Factor	Transcription Factor Class	# of Positive Genes in RWV Set	% of Positive Genes in RWV Set	TFBS Rate in Background Set (%)	TFBS Rate in RWV Set (%)	Z-score	Fisher score
HNF4a	Nuclear Receptor	138	52.9	0.54	0.76	21.9	7.76×10^{-8}
COUPTF1	Nuclear Receptor	88	33.7	0.28	0.38	13.7	2.00×10^{-5}
HNF1a	Homeobox	67	25.7	0.22	0.29	9.9	2.32×10^{-4}
NKX2-5	Homeobox	222	85.1	3.70	3.92	8.5	0.057
C/EBPa	bZIP	162	62.1	1.24	1.36	7.9	0.012
PBX1	Homeobox	60	23.0	0.19	0.23	7.8	0.007
PAX4	Paired-homeobox	6	2.3	0.02	0.04	7.6	0.097
PRRX2	Homeobox	225	86.2	2.29	2.43	6.8	1.29×10^{-4}
FOXQ1	Forkhead	117	44.8	0.60	0.68	6.8	0.009
TLX1-NFIC	Homeobox/CAAT	22	8.4	0.05	0.07	6.1	0.026

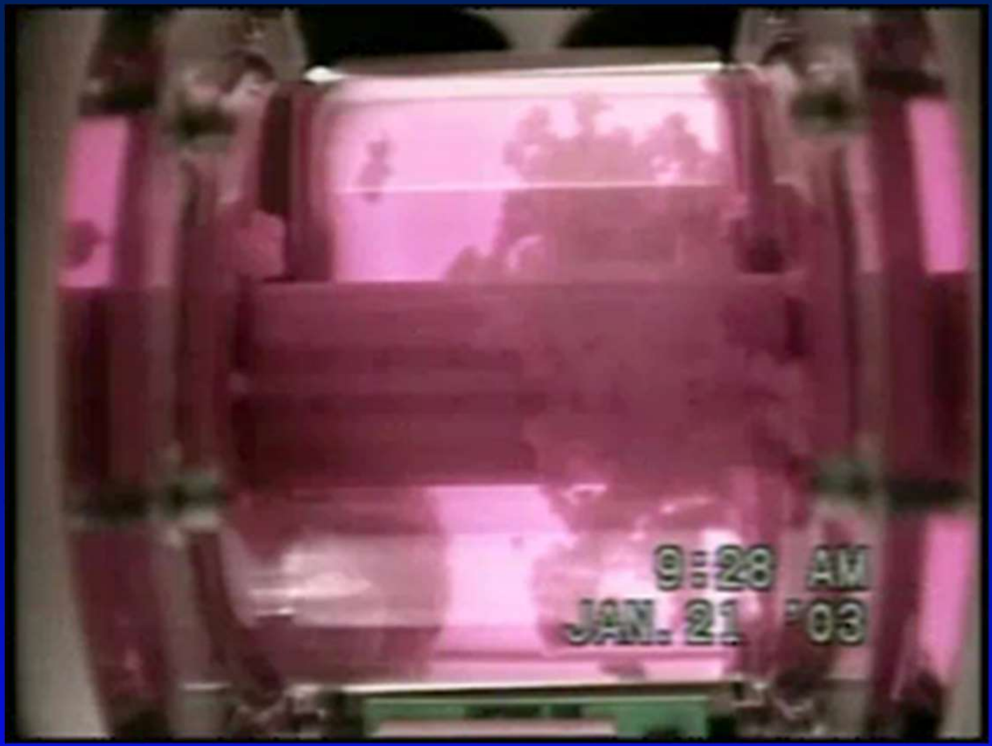
Chang TT and Hughes-Fulford M, Biomaterials, 2014

Forces Acting on Organoids in RWV



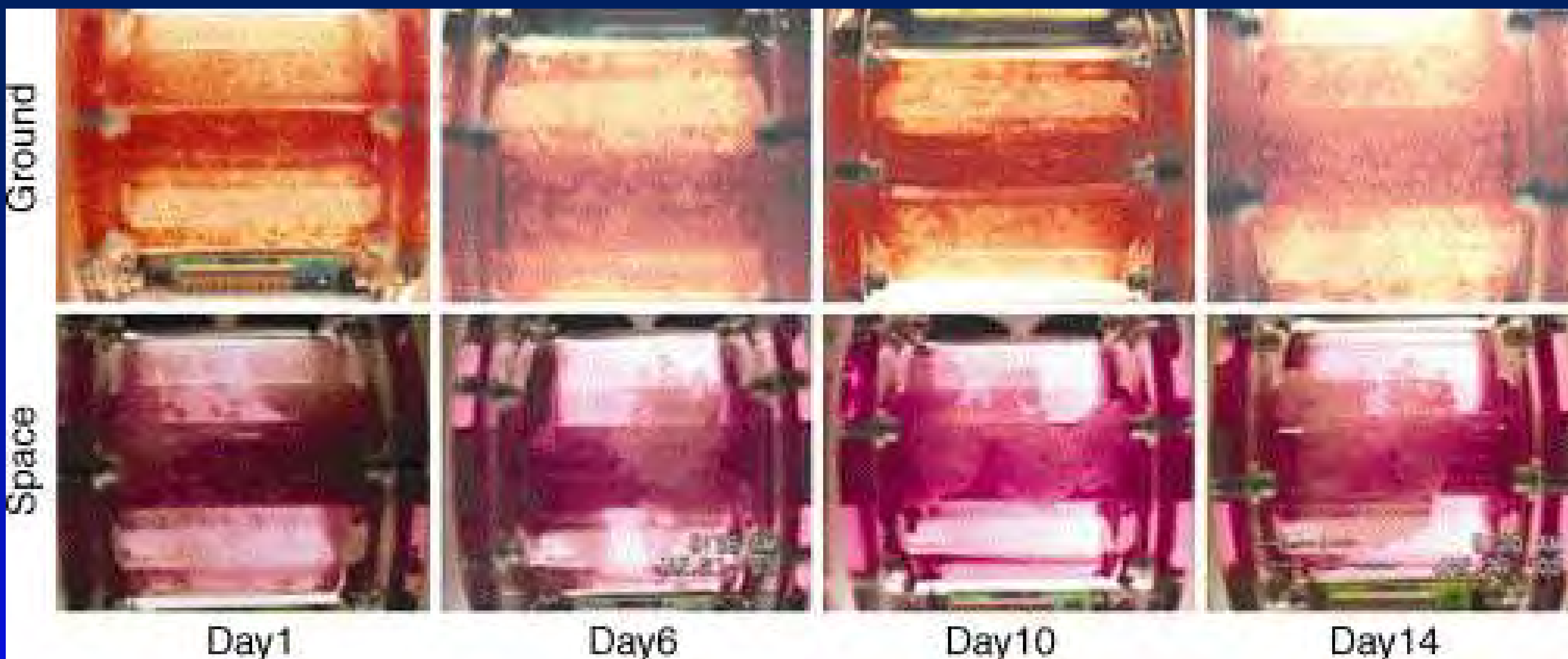
Hammond TG and Hammond JM, Am J Physiol Renal Physiol, 2001

Organoid Formation in Space



Courtesy of NASA/Marshall Space Flight Center
https://archive.org/details/MSFC-0700449

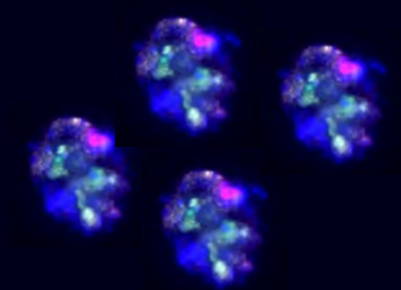
Limitations of RWV
Ground vs. Space



Wang R et al., Semin Cancer Biol, 2005

Liver Tissue Engineering in Space

NSF/CASIS Collaboration on Tissue International Space Station (ISS) to Benefit Life on Earth



Self-Assembly

Pros:

- Emulates biological complexity and structure

Challenges:

- Requires deep understanding of biology
- Question of scale

Tissue Engineering in Space

- There is a critical unmet need
- There remains barriers to creating thick vascularized tissues
- Sustained high-quality microgravity may be a key tool

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Macarena Lolas
Manuel Armas-Phan
Miya Yoshida
Tristan Bond
Tanner Barnes

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