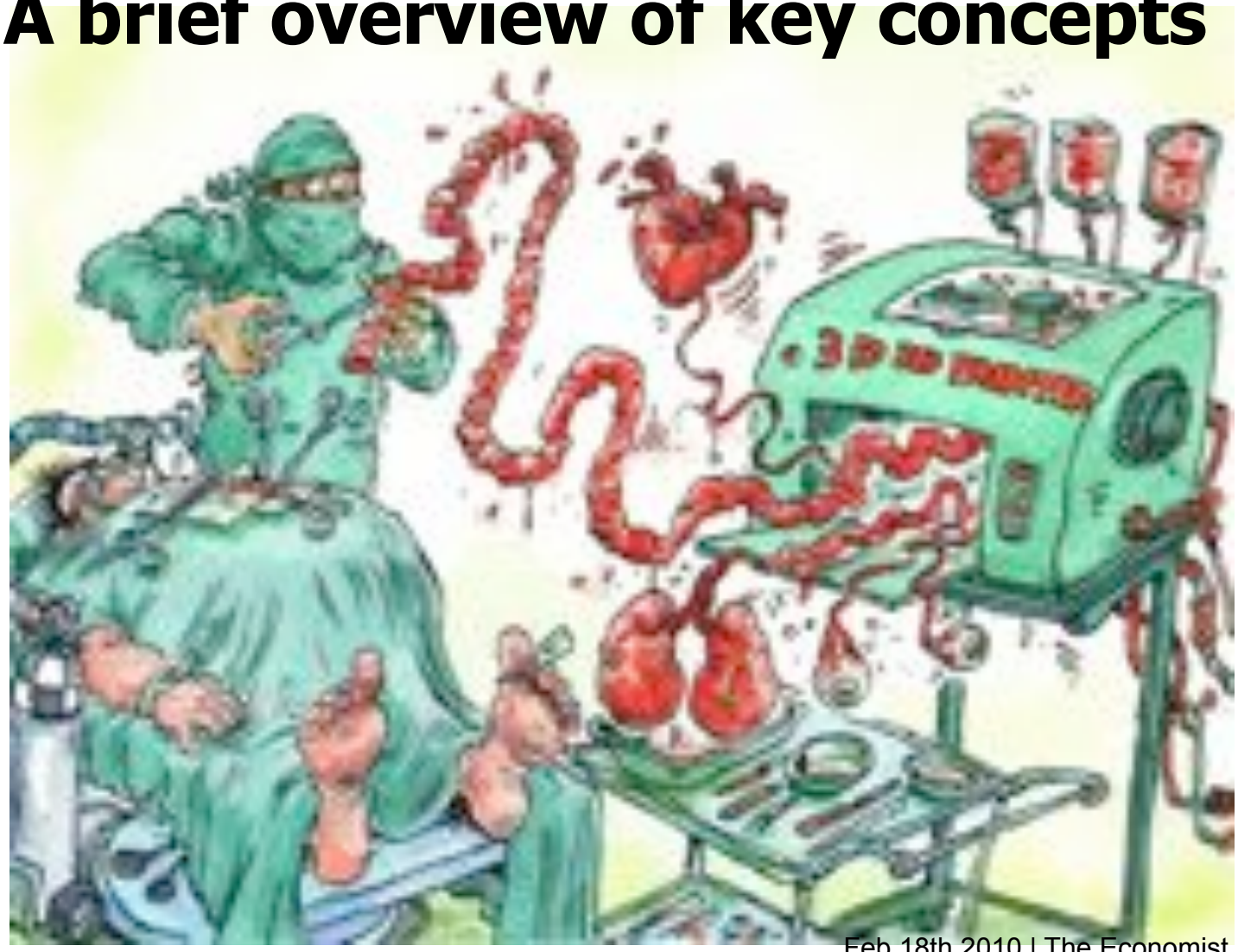
Regenerative Medicine:
A brief overview of key concepts

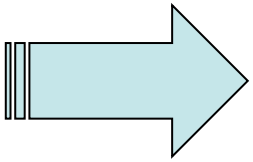


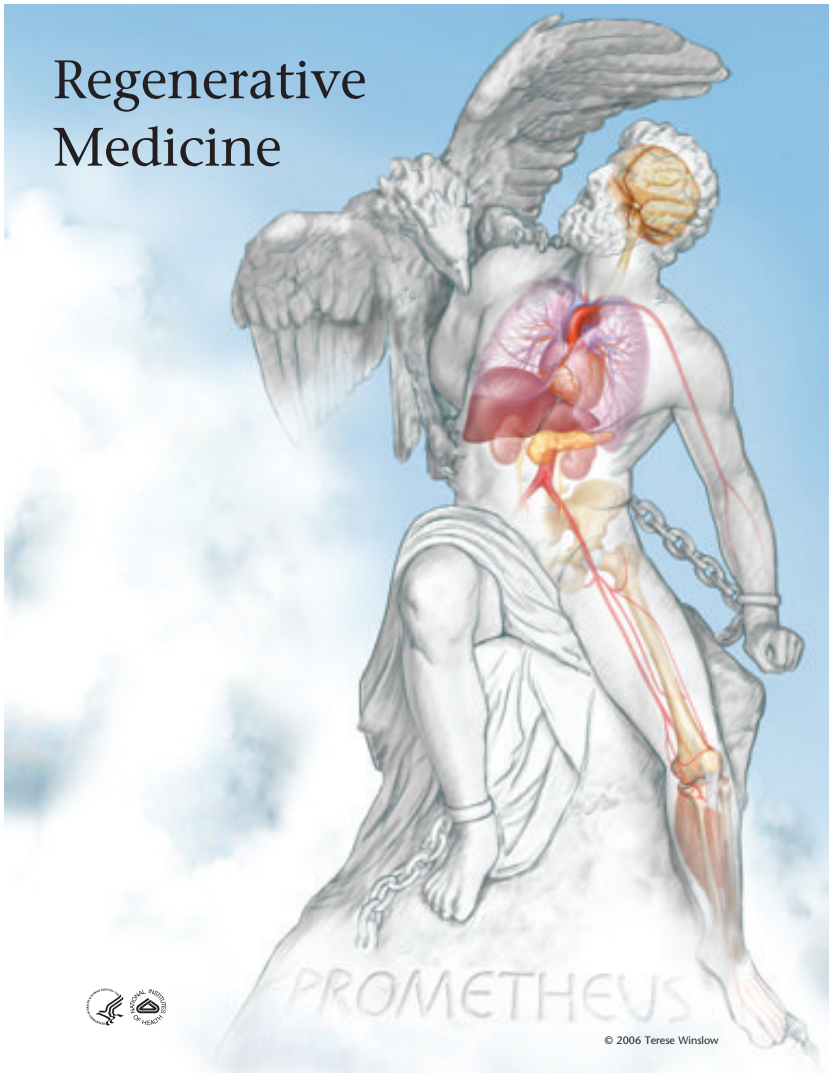
Feb 18th 2010 | The Economist

Matthias Lutolf, Institute of Bioengineering, EPFL

‘Optimal and sub-optimal regenerators’

http://regeneration.bio.uci.edu/content.html





Cells outperform devices!

Overview

1. Tissue Engineering 1.0: the beginning

2. *Ex situ* versus *in situ* tissue engineering

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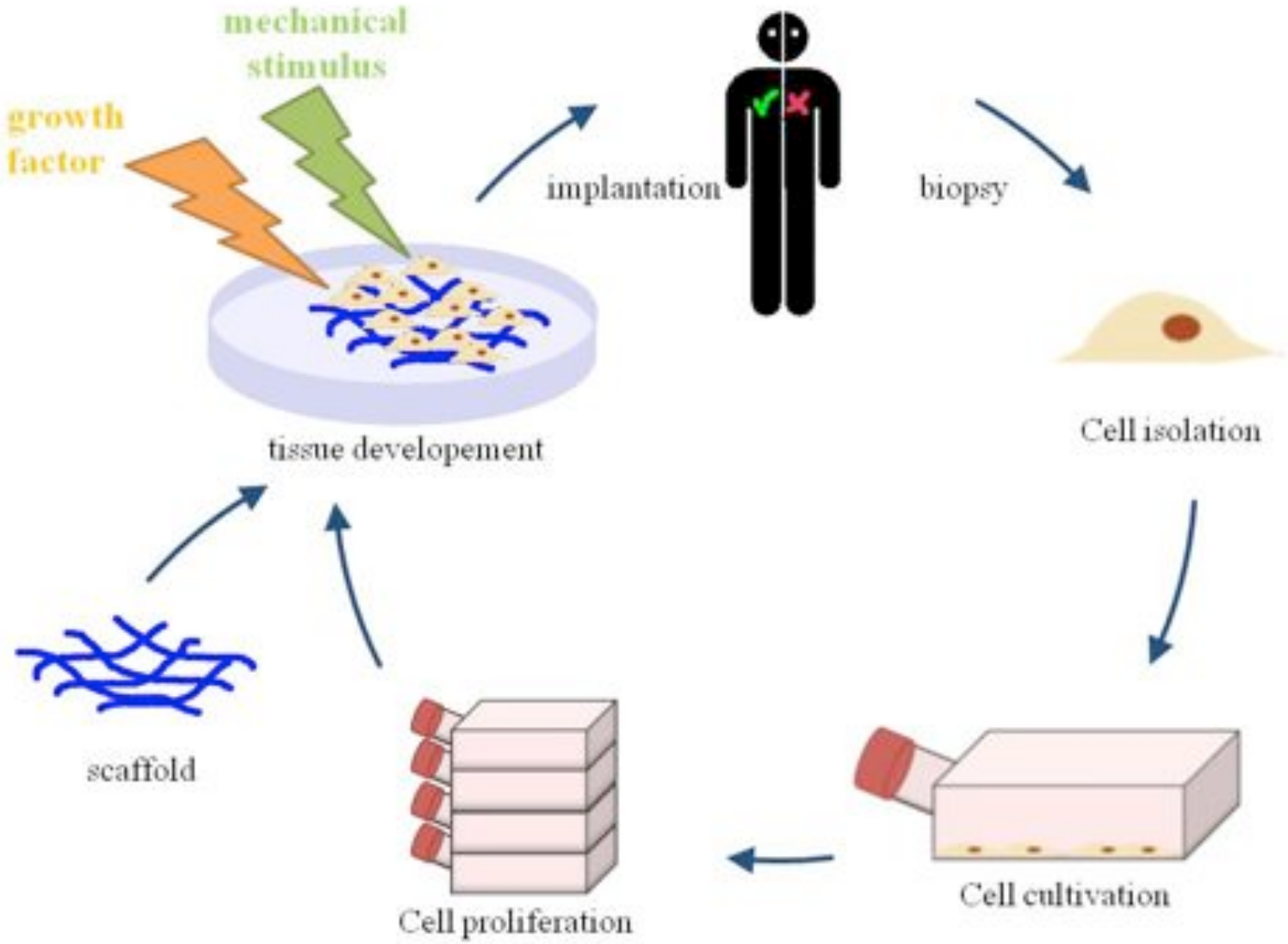
Tissue Engineering v1.0



Wikipedia

WO 1988003785 A1
Chimeric neomorphogenesis of organs by controlled cellular implantation using artificial matrices
Filed 20 Nov 1987

Cao, Y.; Vacanti, J. P.; Paige, K. T.; Upton, J.; Vacanti, C. A. (1997). "Transplantation of chondrocytes utilizing a polymer-cell construct to produce tissue-engineered cartilage in the shape of a human ear". Plastic and reconstructive surgery 100 (2): 297–302

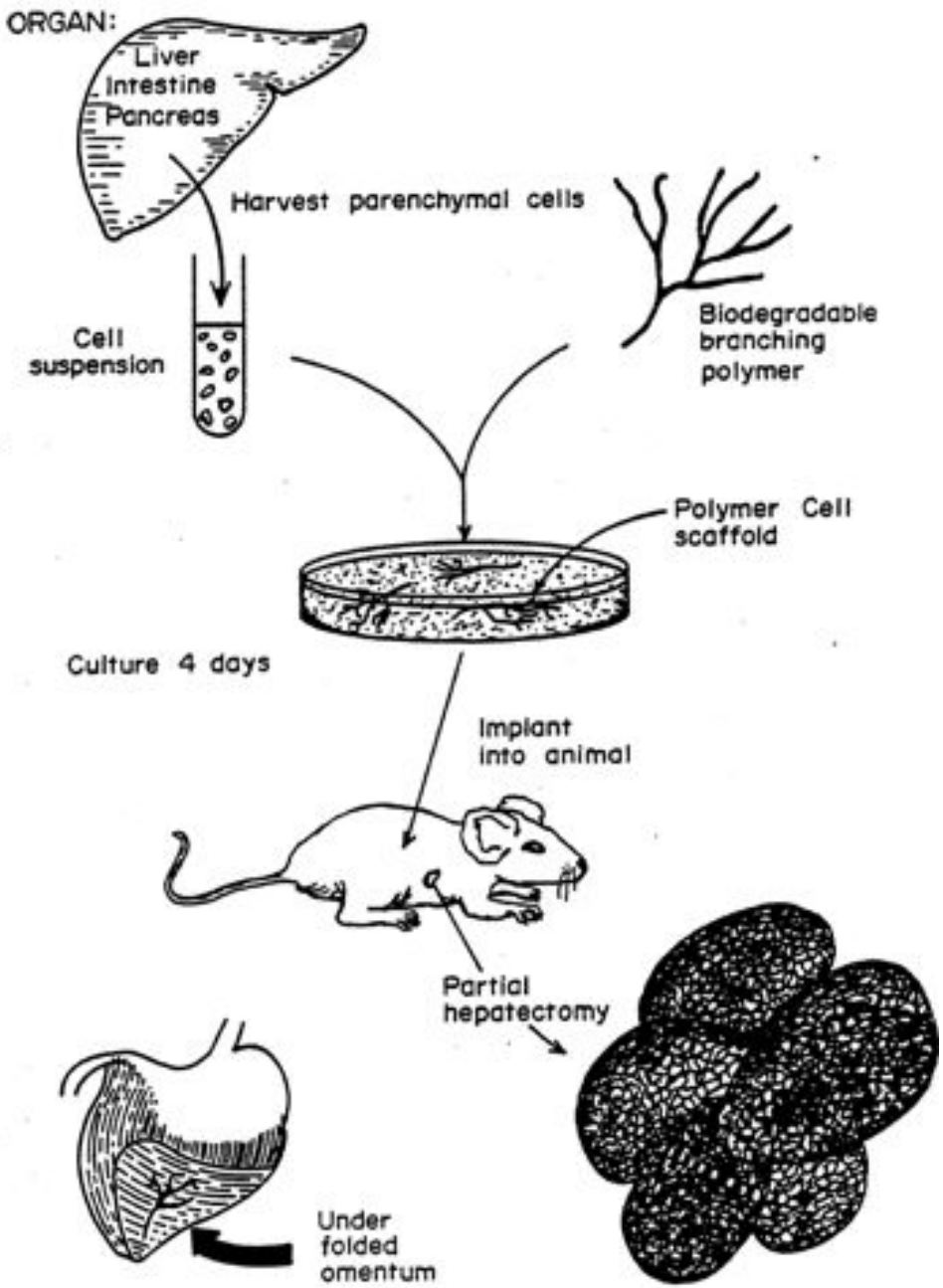
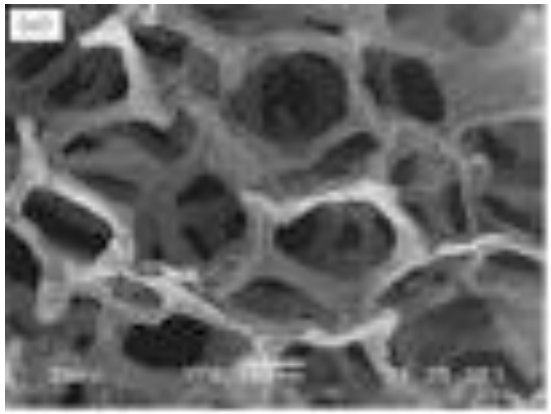
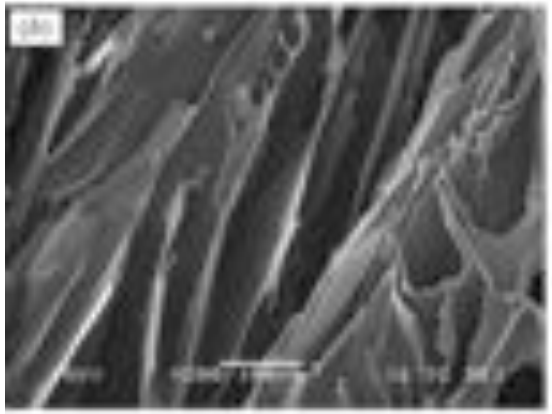
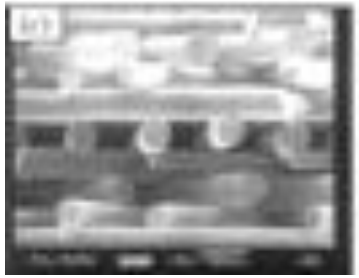


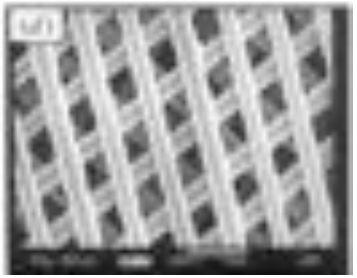
FIG.I

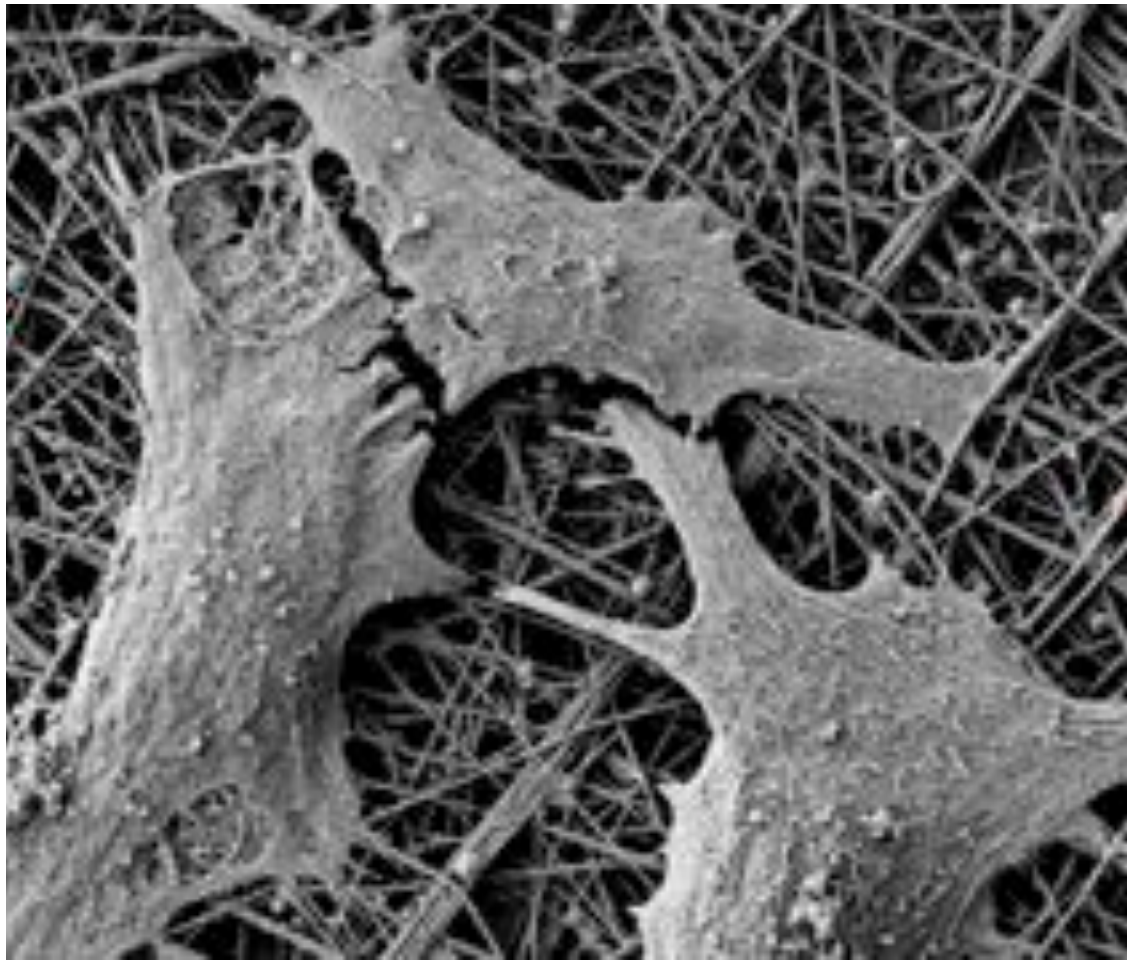
The scaffolds: not quite like native extracellular matrix…











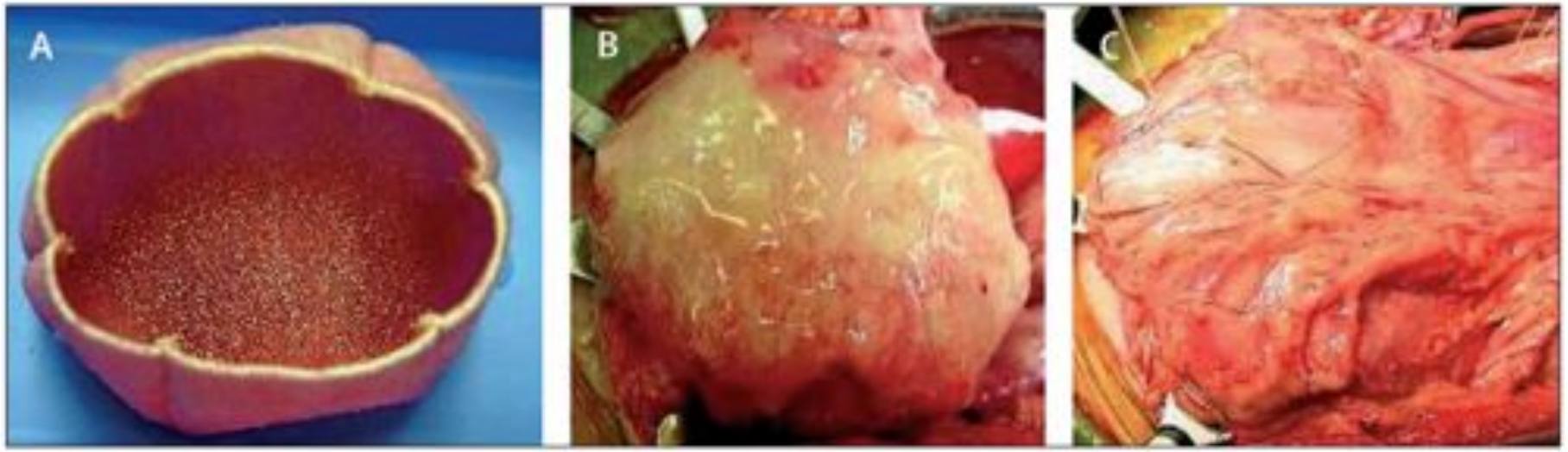


Figure 1: **Construction of engineered bladder**
Scaffold seeded with cells (A) and engineered bladder anastamosed to native bladder with running 4–0 polyglycolic sutures (B). Implant covered with fibrin glue and omentum (C).

Tissue-engineered autologous bladders for patients needing cystoplasty

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

Summary

Background Patients with end-stage bladder disease can be treated with cystoplasty using gastrointestinal segments. The presence of such segments in the urinary tract has been associated with many complications. We explored an alternative approach using autologous engineered bladder tissues for reconstruction.

Methods Seven patients with myelomeningocele, aged 4–19 years, with high-pressure or poorly compliant bladders, were identified as candidates for cystoplasty. A bladder biopsy was obtained from each patient. Urothelial and muscle cells

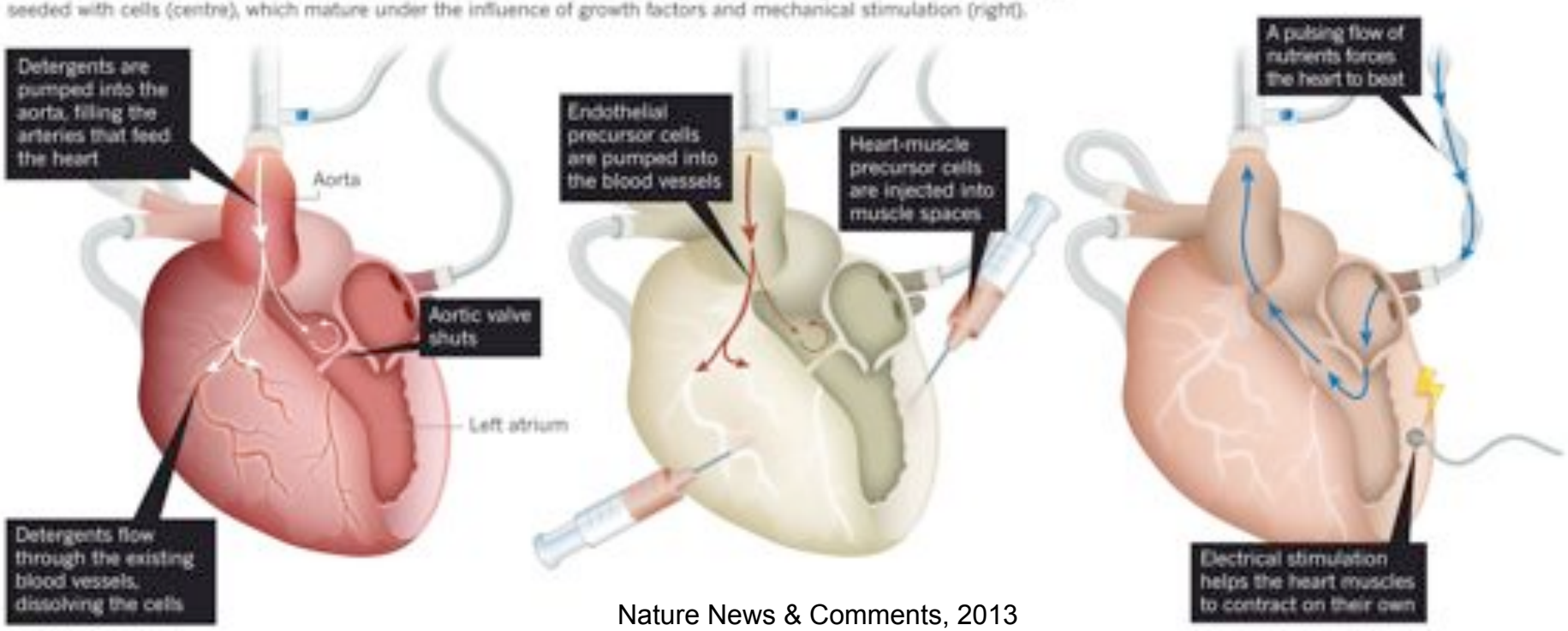
Lancet 2006; 367: 1241–46
Published Online
April 4, 2006
DOI:10.1016/S0140-6736(06)68438-9

See Comment page 1215

*** very complicated & expensive**
*** scaffold degradation products cause inflammation & necrosis**

CUSTOMIZED ORGANS

To construct a new heart, researchers first remove all cells from a donor organ (left), leaving a protein scaffold. That is seeded with cells (centre), which mature under the influence of growth factors and mechanical stimulation (right).



Nature News & Comments, 2013

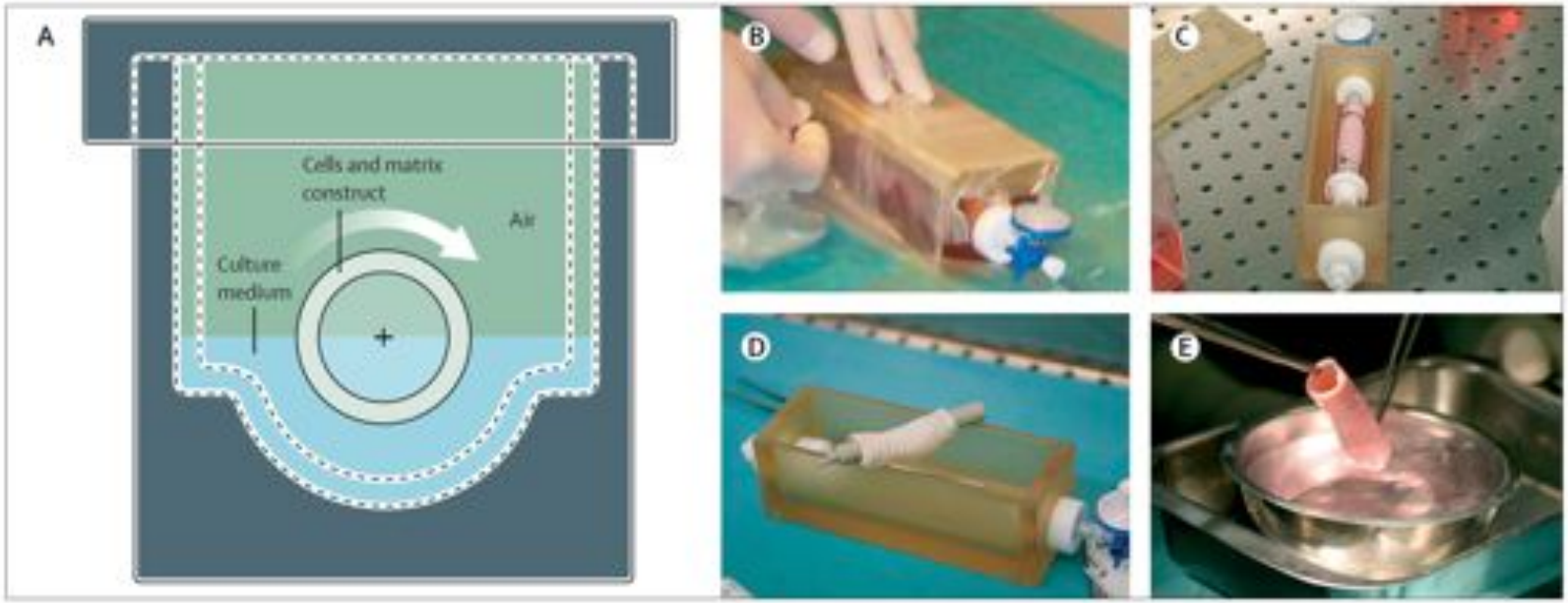


Figure 2: Bioreactor developed for airway tissue engineering
(A) Schematic lateral view, highlighting the rotation of the matrix around its longitudinal axis. The design has separate compartments for lumen and outer surface, and is regularly rotated through a motor to apply the sheer stress needed for growth, distribute nutrients and waste, and ensure even exposure to applied cells. (B) The sealed device. (C) Bioreactor with the graft in situ. (D) Bioreactor after removal of the graft. (E) The final graft immediately before surgical implantation.

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

Summary

Background The loss of a normal airway is devastating. Attempts to replace large airways have met with serious problems. Prerequisites for a tissue-engineered replacement are a suitable matrix, cells, ideal mechanical properties, and the absence of antigenicity. We aimed to bioengineer tubular tracheal matrices, using a tissue-engineering protocol, and to assess the application of this technology in a patient with end-stage airway disease.

Lancet 2008; 372: 2023–30
Published Online November 19, 2008
DOI:10.1016/S0140-6736(08)61598-6

*** Still complicated & expensive**

Overview

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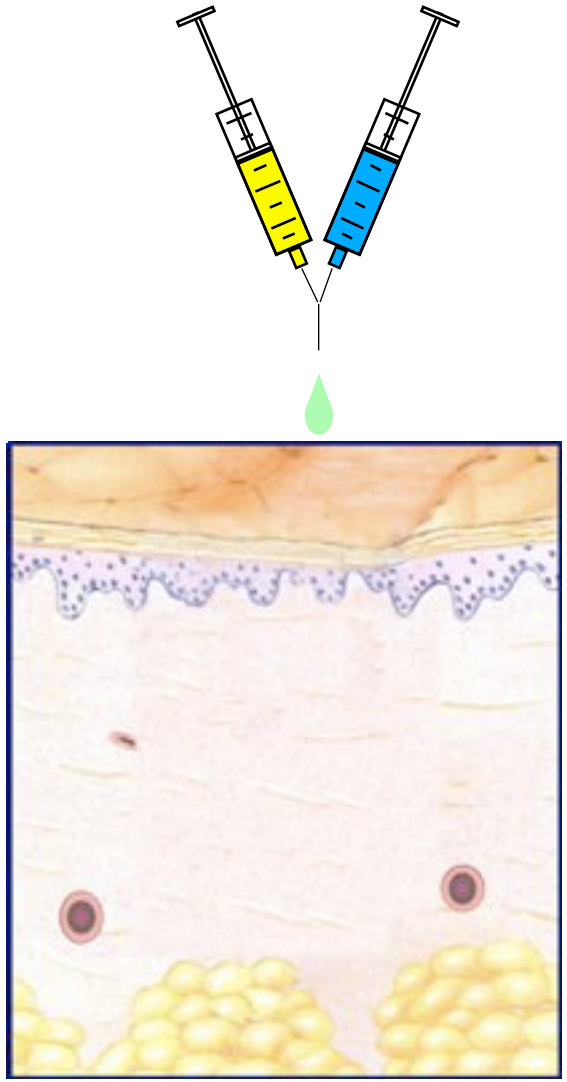
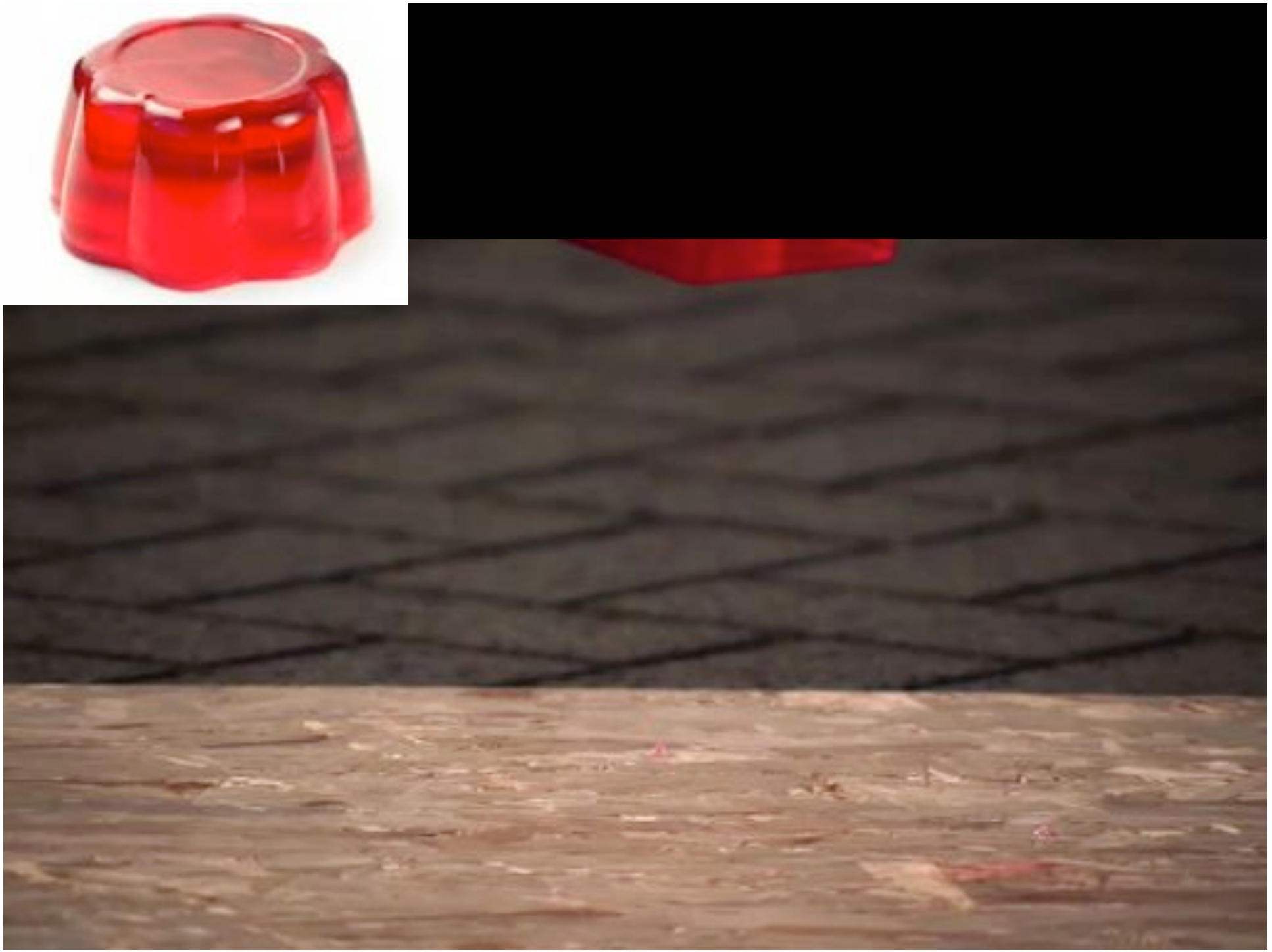
In situ tissue engineering: smart drug delivery



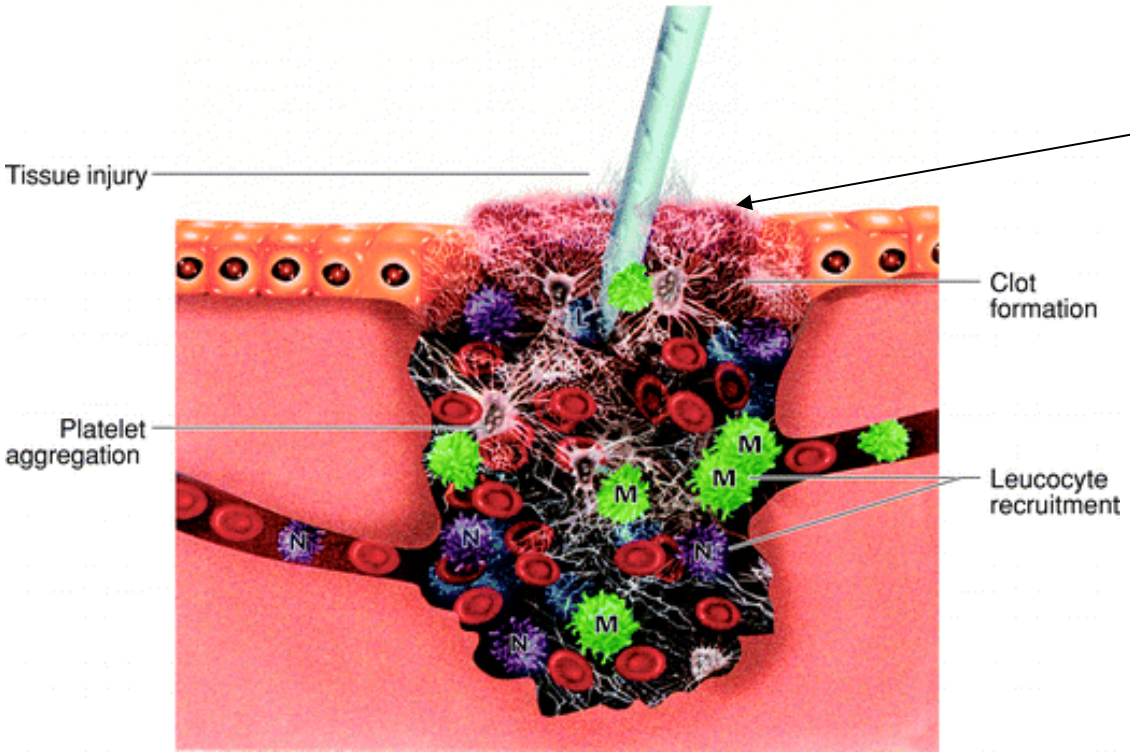
Stimulating self-repair

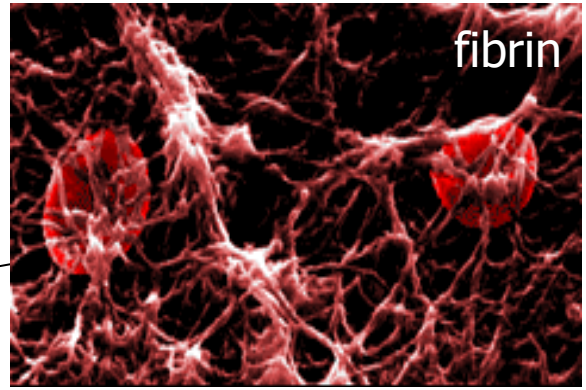
- In situ formation of bioactive matrix
- Attraction of regeneration-competent cells (stem/progenitor cells)
- Cell infiltration
- Controlled matrix degradation/ remodeling
- Progenitor cell differentiation into tissue-specific cells; tissue regeneration

Hubbell Lab, ETH Zurich, 90's +



Fibrin: Nature's regeneration matrix





A temporary viscoelastic gel as scaffold for invading cells that

- forms *in situ*
- is cell-adhesive
- is proteolytically degradable (mainly via matrix metalloprot.)
- stores and releases protein growth factors

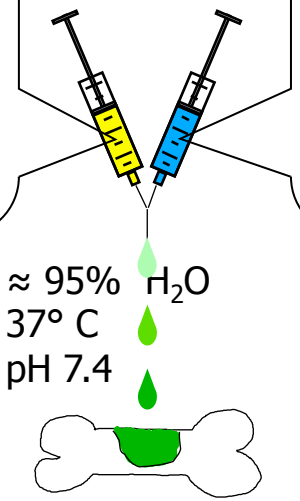
‘Synthetic fibrin’ for localized morphogen delivery

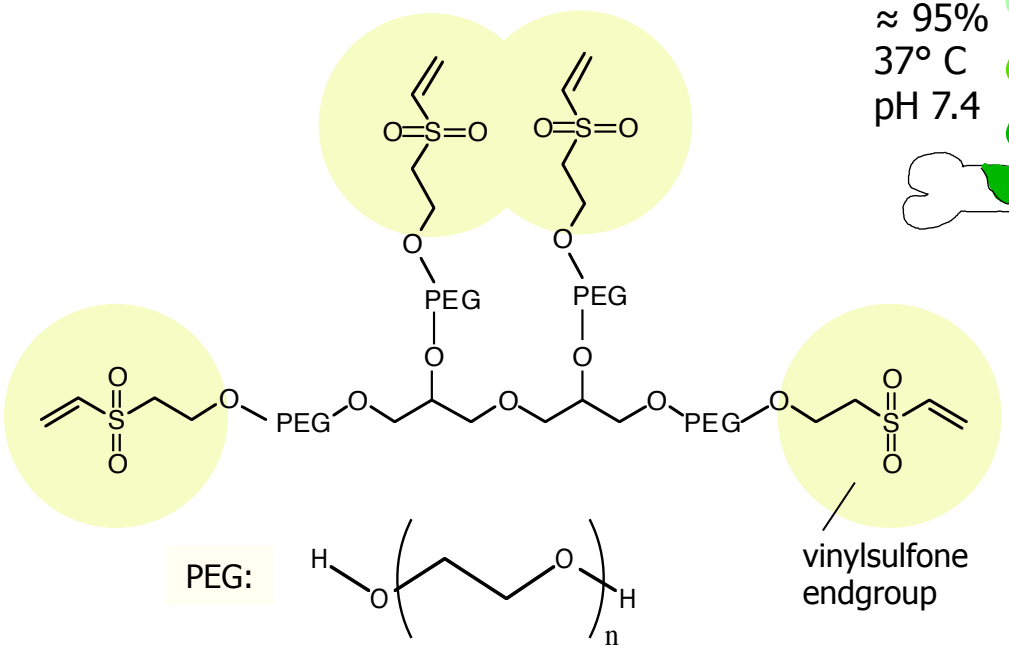
A: Poly(ethylene glycol)

- hydrophilic, „biocompatible“ (inert)
- cross-linker (functionality > 2)
- structural properties

B: Oligopeptides

- „biological information“
- cell-responsiveness (protease sensitivity)





+

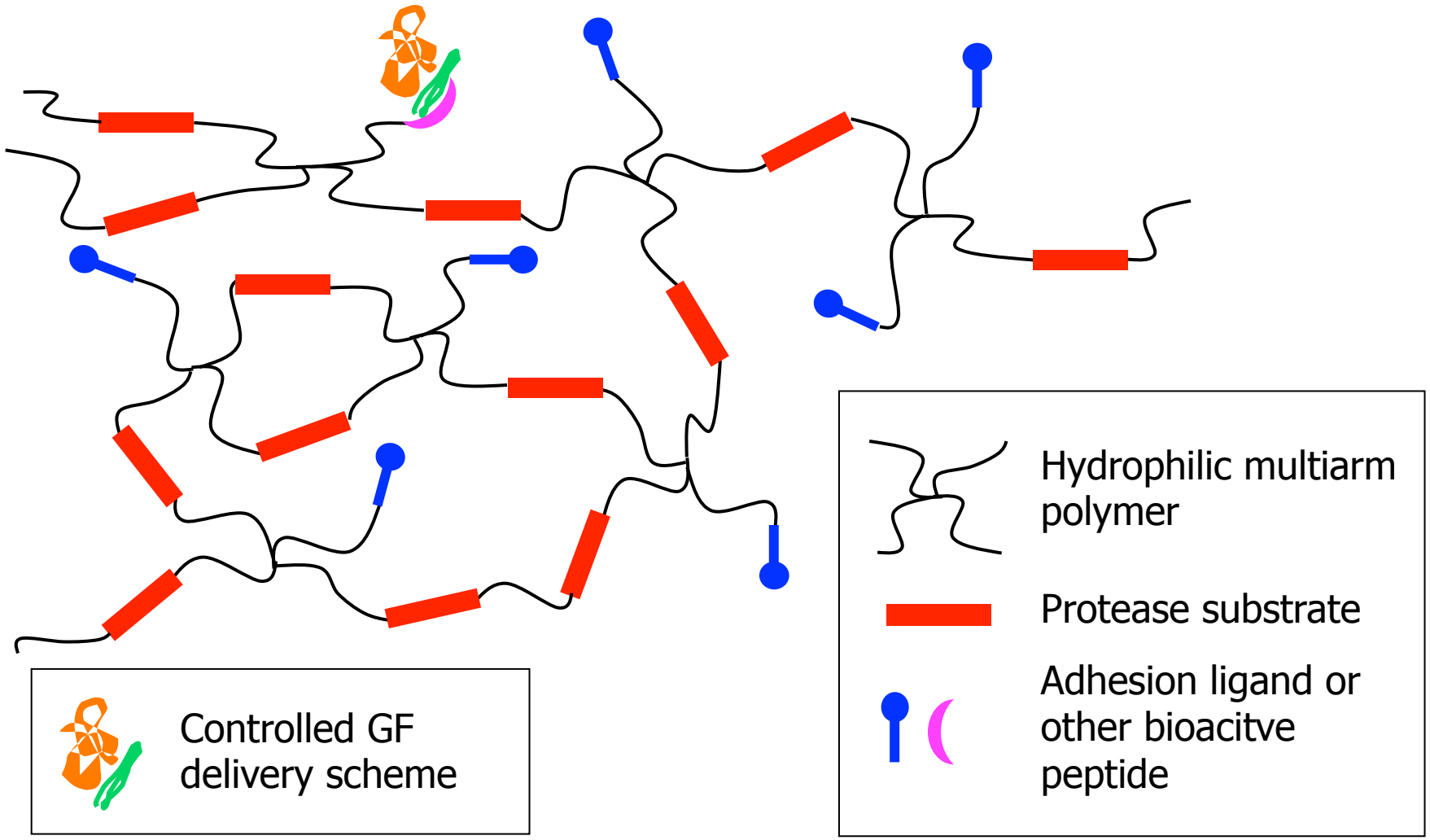
SH
C adhesion ligand

e.g. Cys-RGDSP

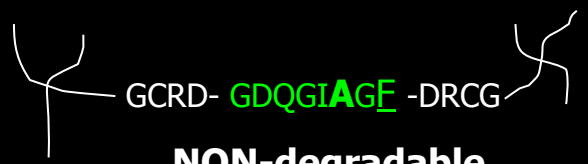
e.g. Cys-GPQG↓IWGQ-Cys

SH
C protease substrate C
SH

Schematic of synthetic hydrogels mimicking the biological key functions of native ECMs



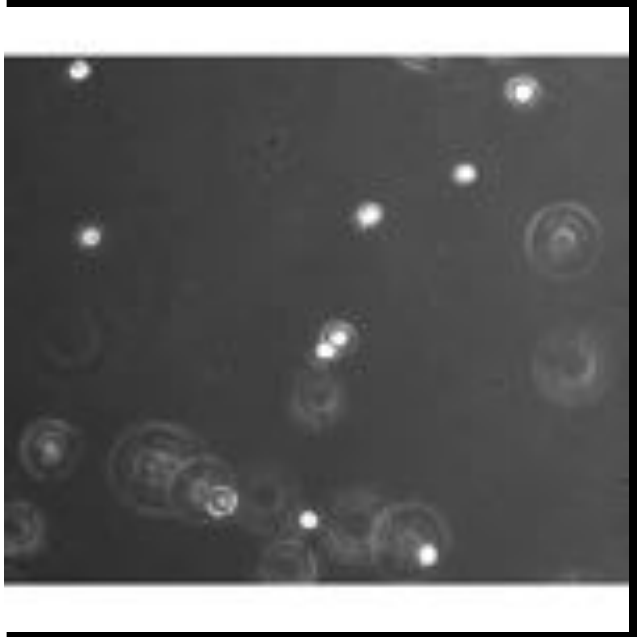
3D cell migration within synthetic ECMs can be controlled by tuning gel degradation kinetics (via k_{cat})

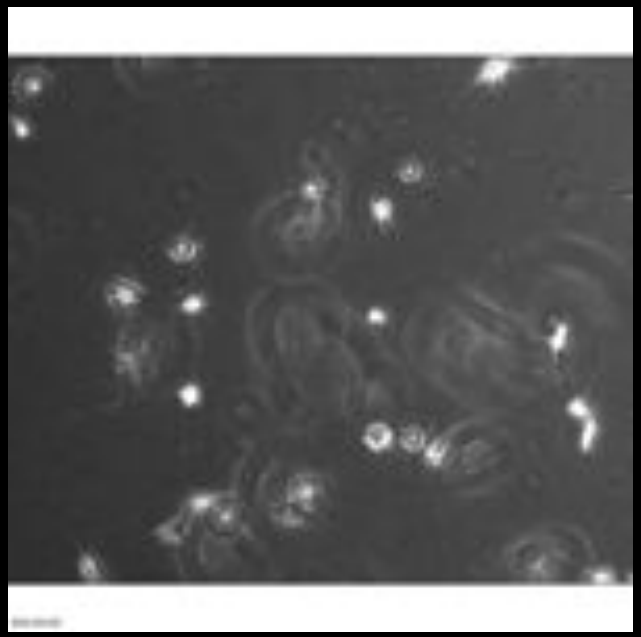


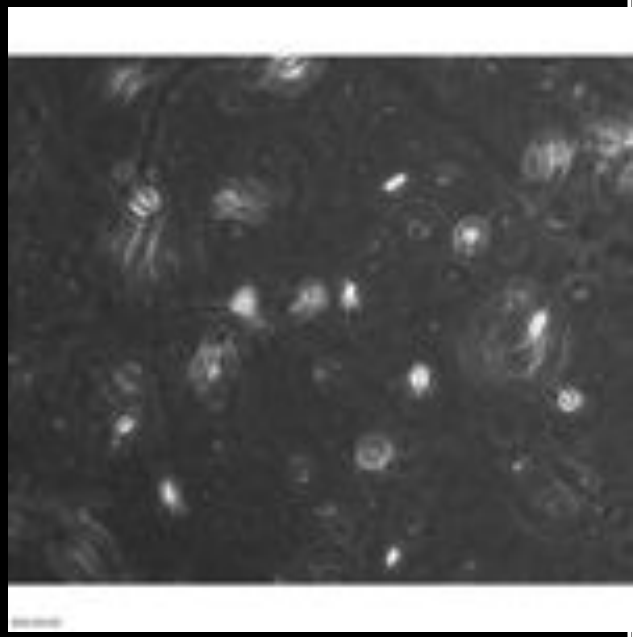
NON-degradable

Natural sequence

Fast degradable

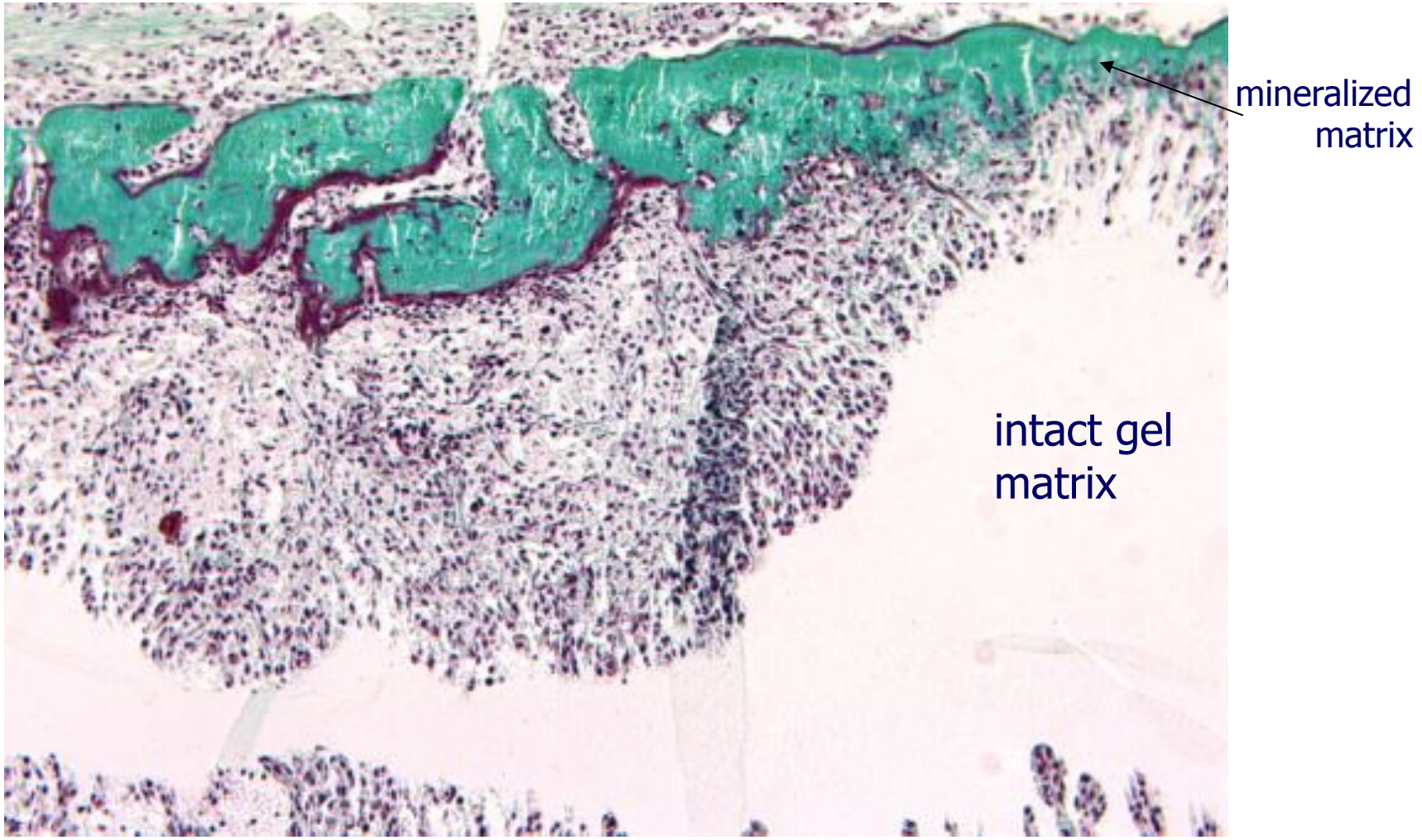




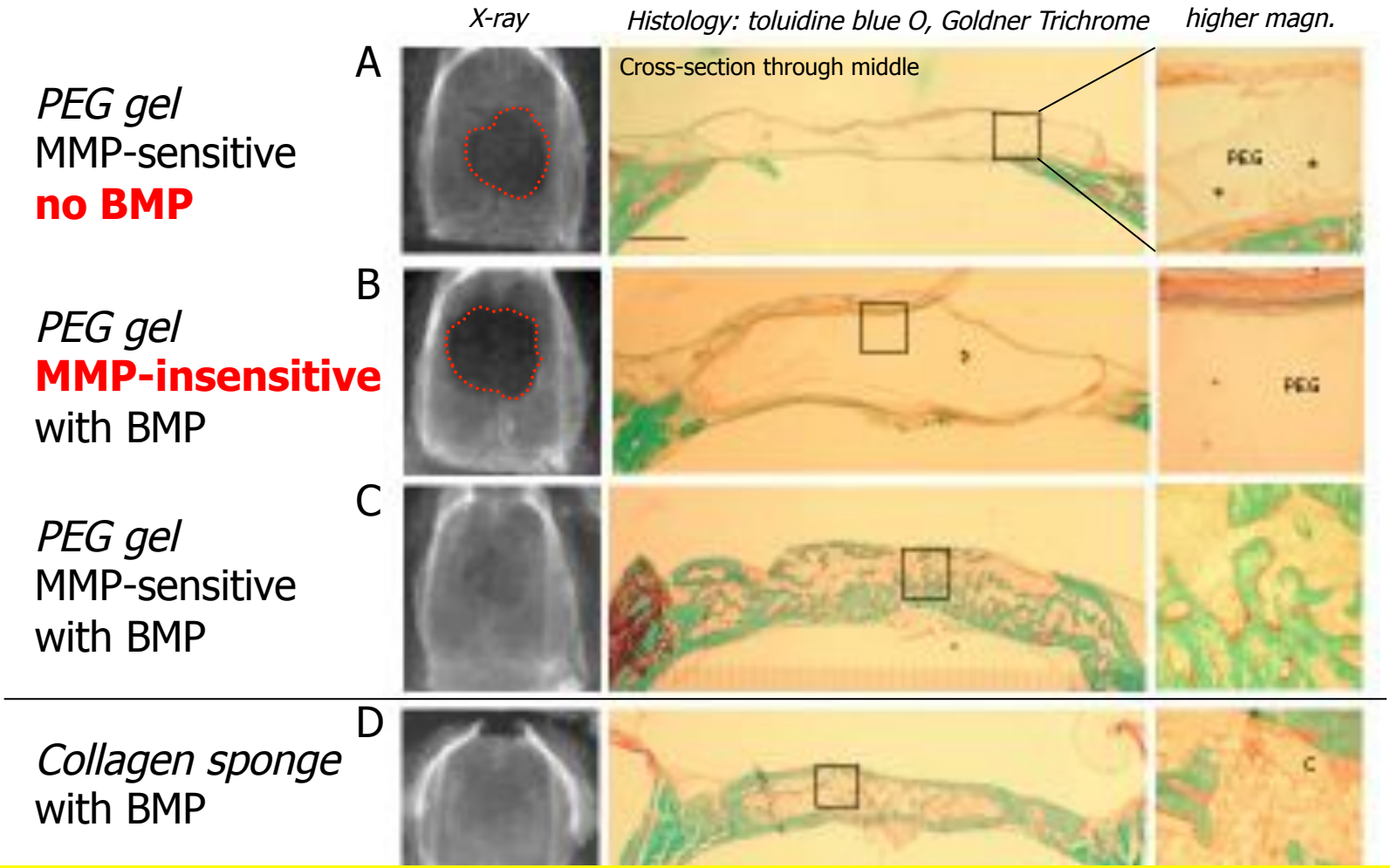


E.g.: Single mesenchymal progenitor cells embedded in gels bearing cell-adhesive and matrix metalloprotease-sensitive building blocks

Endogenous (stem/progenitor) cell invasion and bone regeneration upon gel remodeling after 3 weeks



Complete gel remodeling and regeneration after 5 weeks



*** limited to some tissues and relatively small defects**

By and large, 'classical' tissue engineering strategies have not fulfilled the huge expectations (scientifically and economically)

The paradigm shift:

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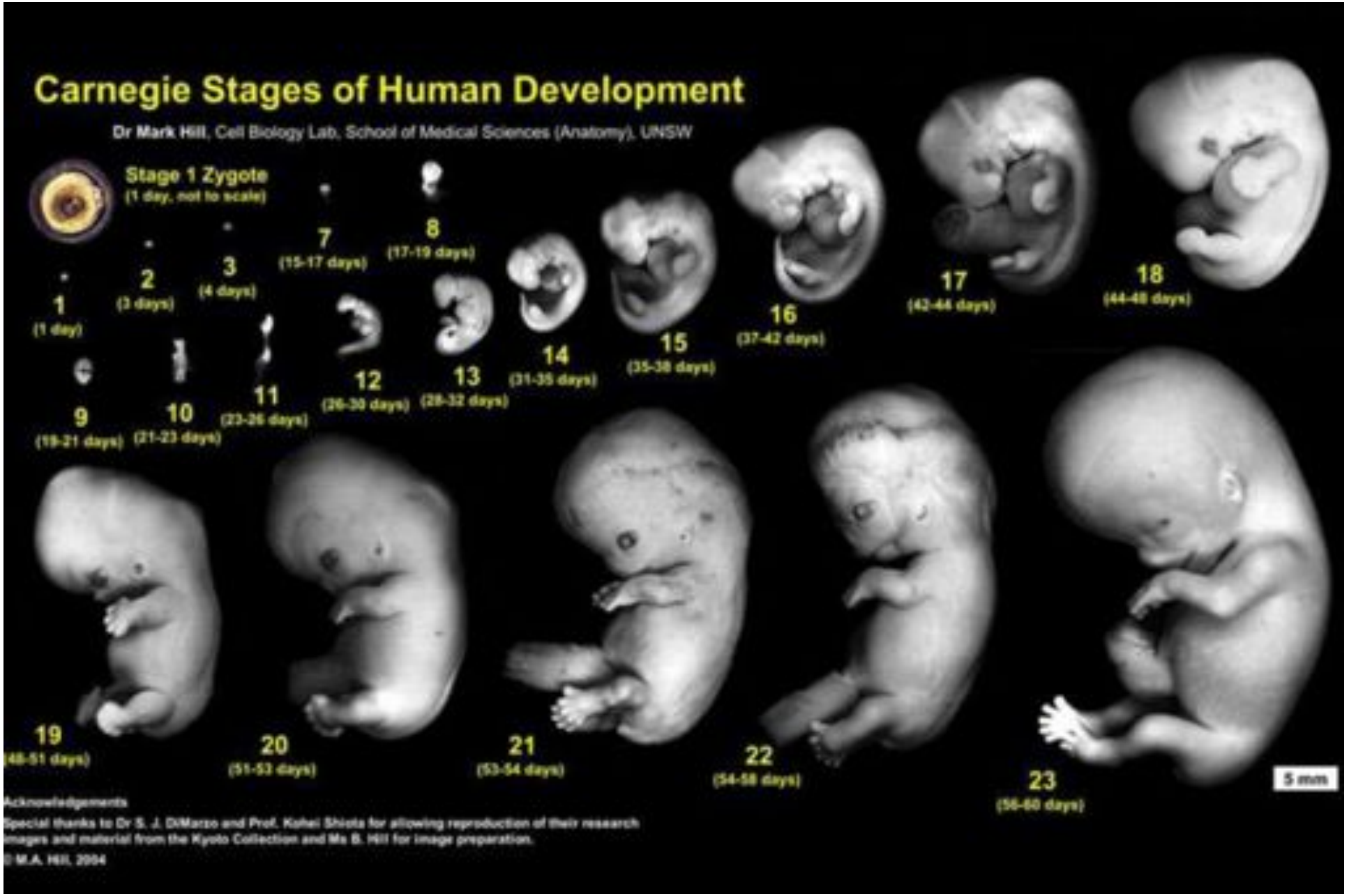
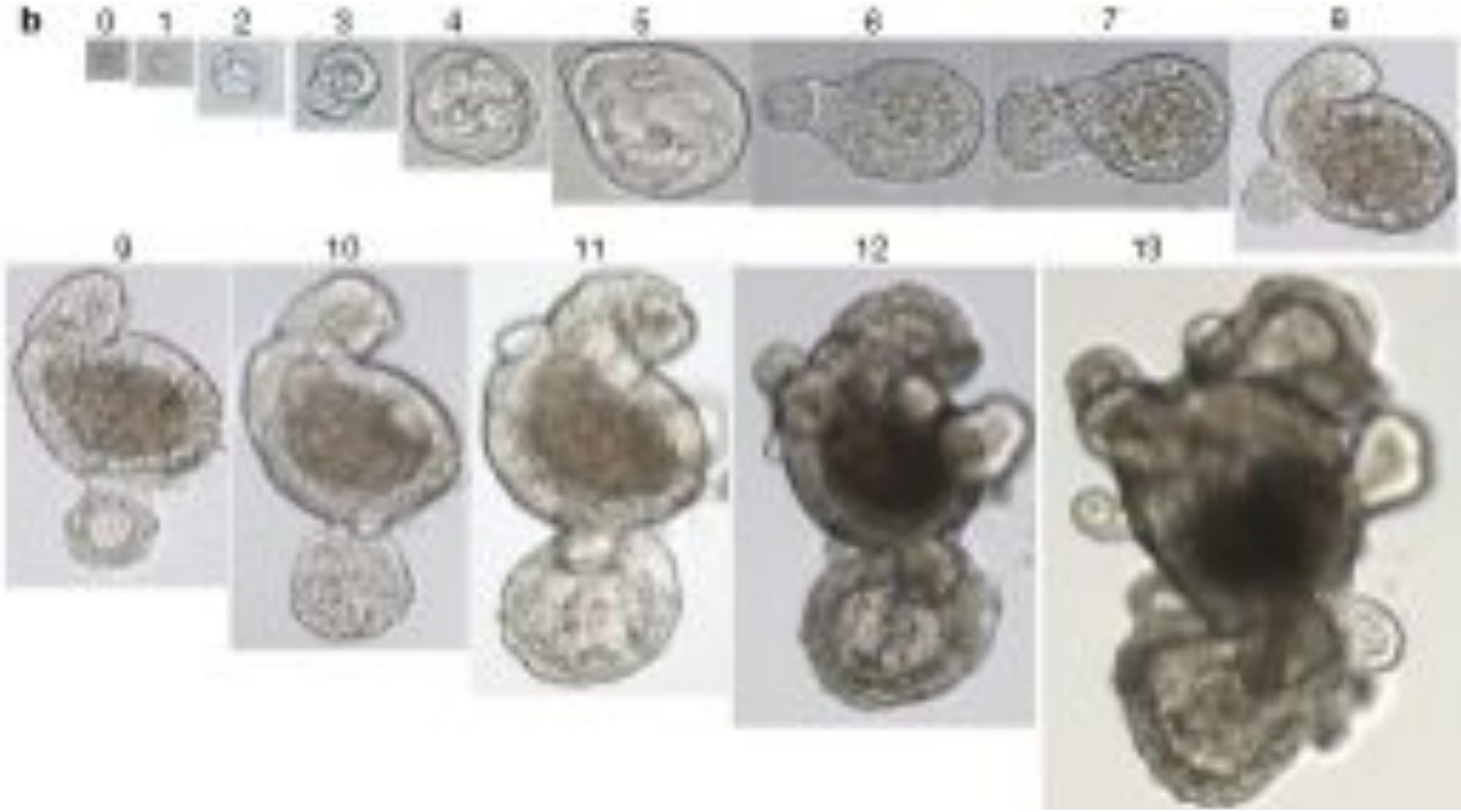
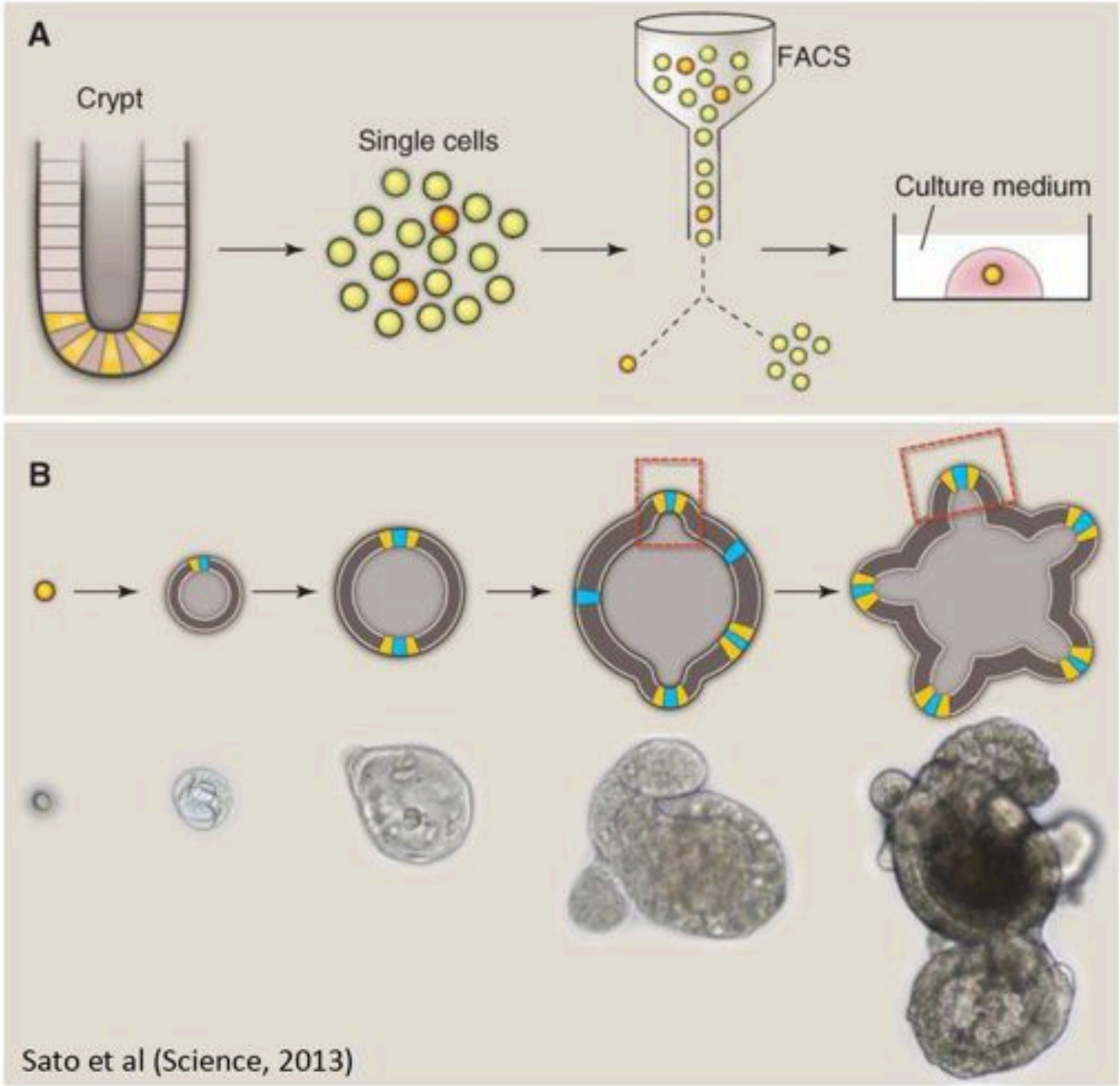
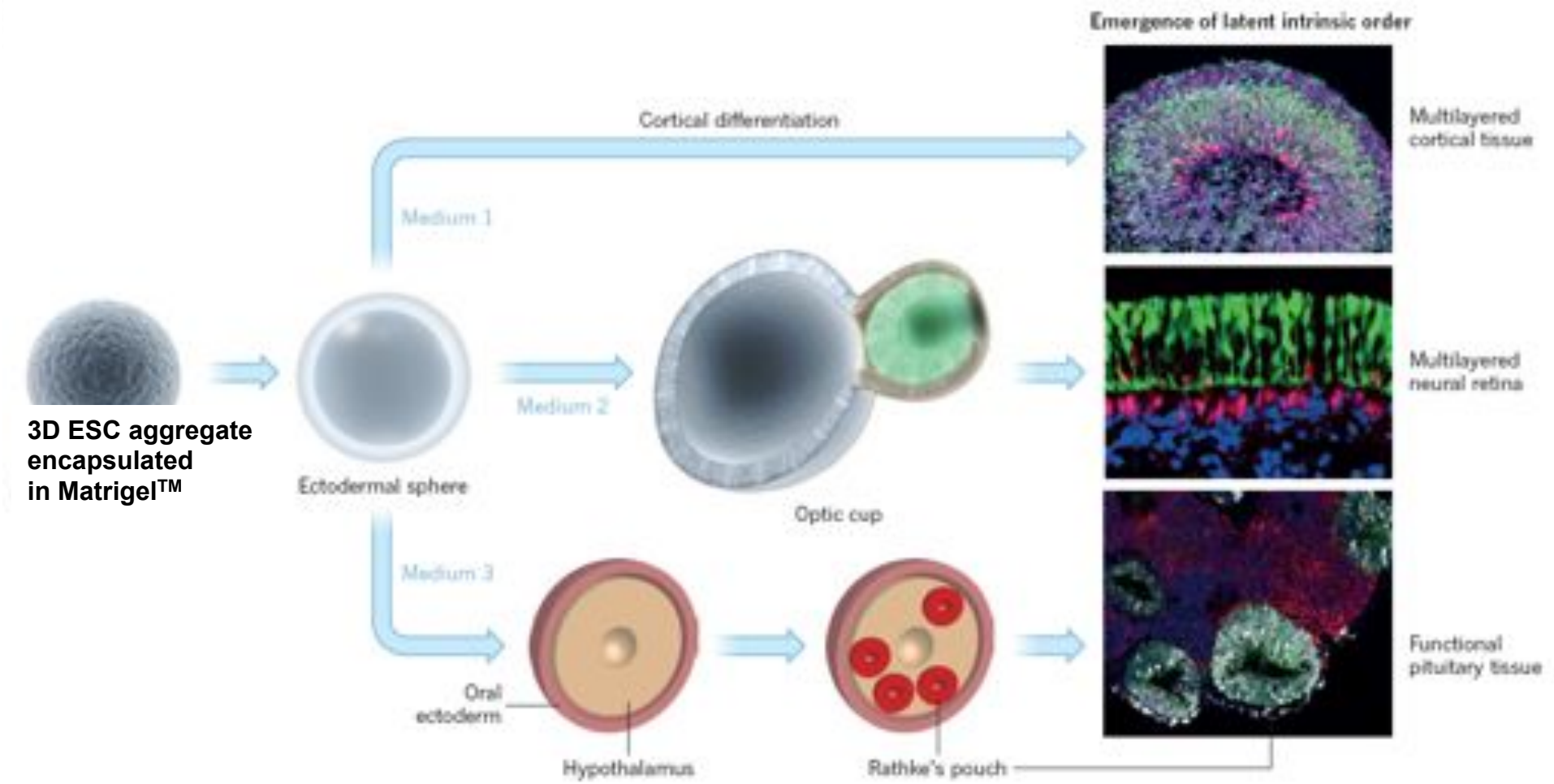

Carnegie Stages of Human Development
Dr Mark Hill, Cell Biology Lab, School of Medical Sciences (Anatomy), UNSW
Stage 1 Zygote
(1 day, not to scale)
1
(1 day)
2
(3 days)
3
(4 days)
7
(15-17 days)
8
(17-19 days)
9
(19-21 days)
10
(21-23 days)
11
(23-26 days)
12
(26-30 days)
13
(28-32 days)
14
(31-35 days)
15
(35-38 days)
16
(37-42 days)
17
(42-44 days)
18
(44-48 days)
19
(48-51 days)
20
(51-53 days)
21
(53-54 days)
22
(54-58 days)
23
(56-60 days)
5 mm
Acknowledgements
Special thanks to Dr S. J. DiMarzo and Prof. Kohei Shiota for allowing reproduction of their research images and material from the Kyoto Collection and Ms B. Hill for image preparation.
© M.A. Hill, 2004



Sato et al, Nature, 2009

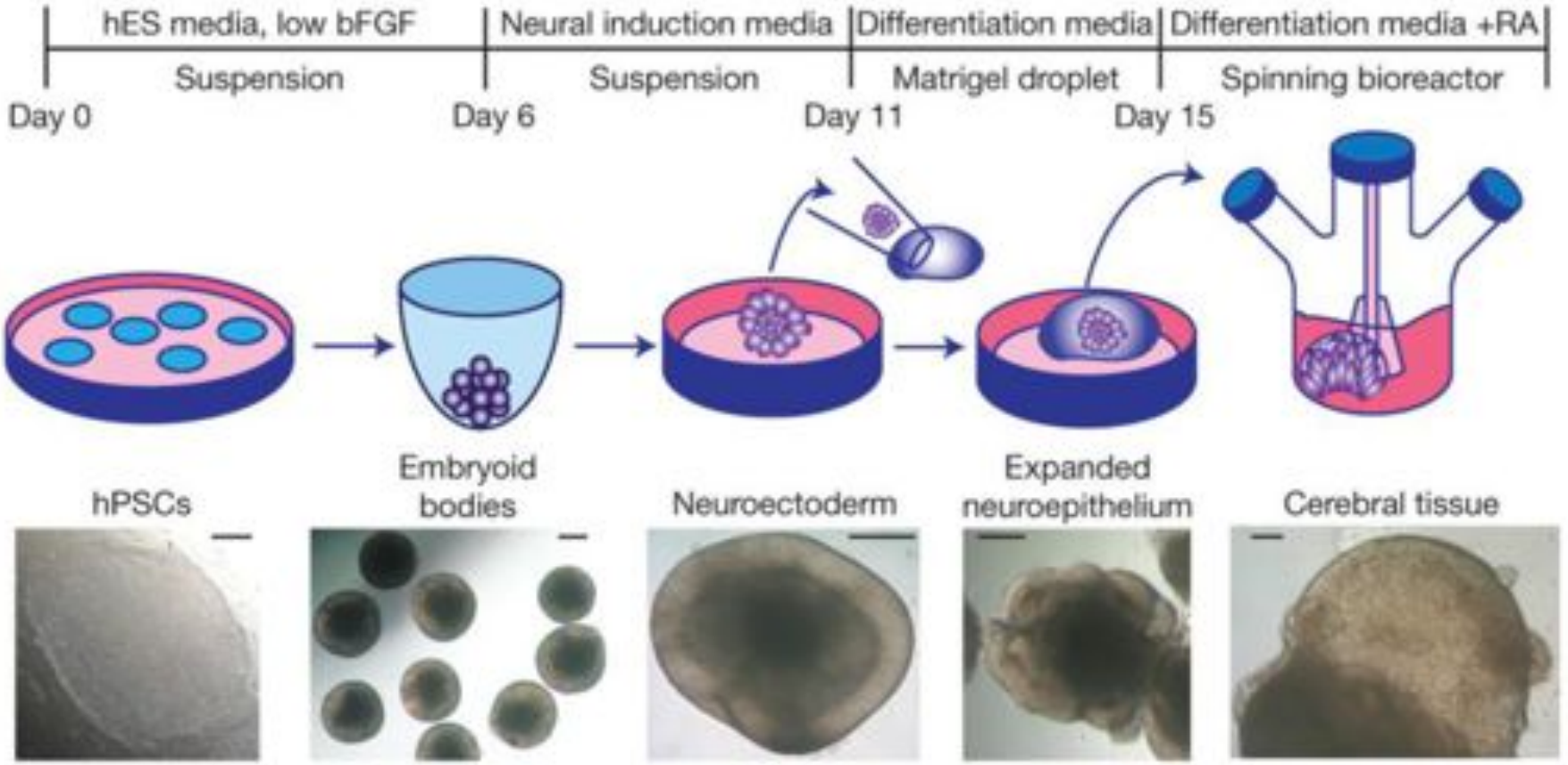

A
Crypt
Single cells
FACS
Culture medium
B
Sato et al (Science, 2013)

Tissue formation by pluripotent stem cell self-organization



Yoshiki Sasai, Nature, 2013

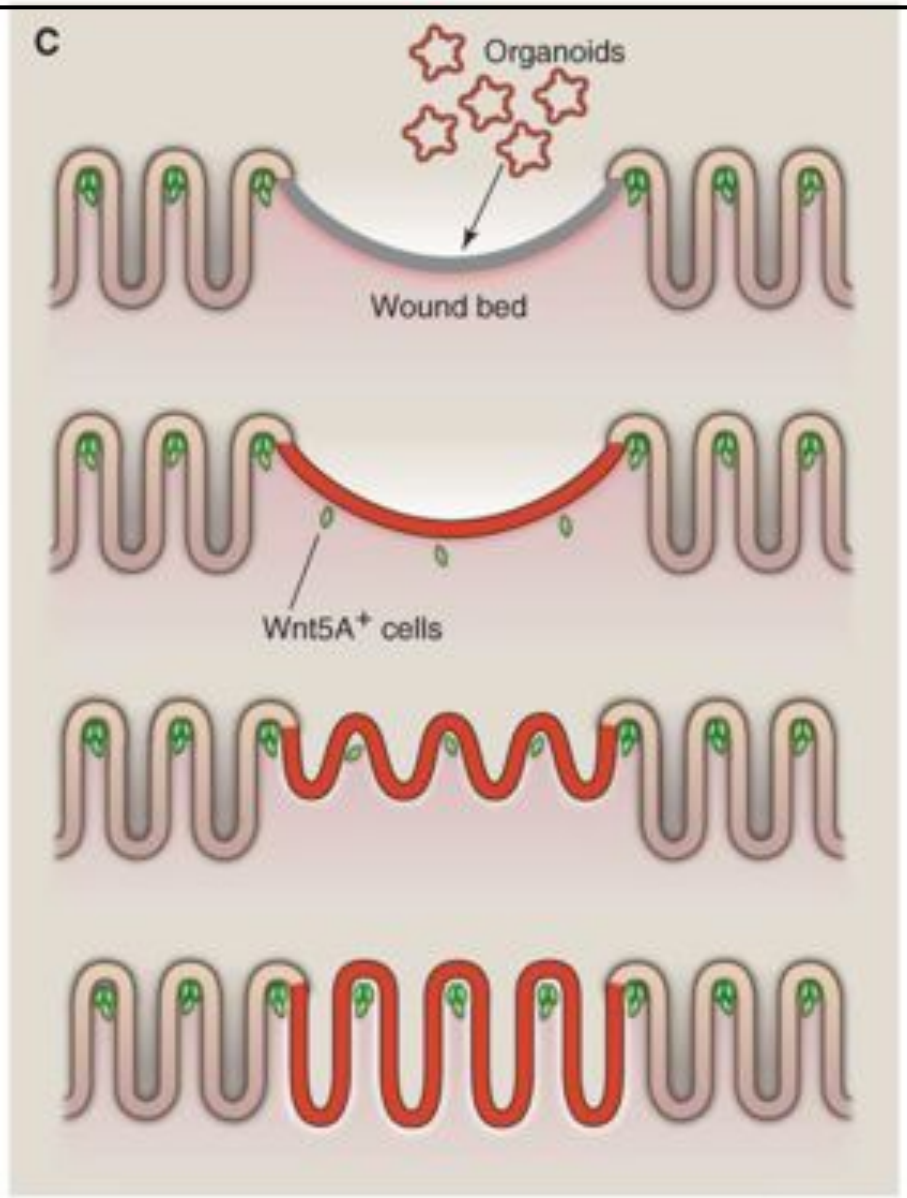
A human brain in a dish…?!

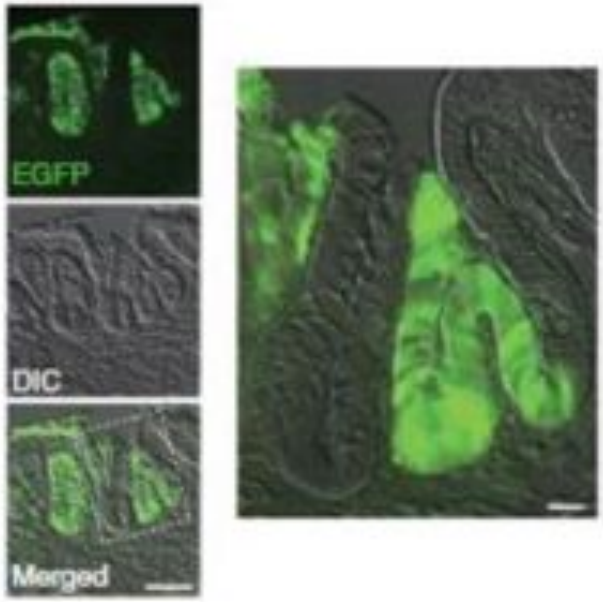


Lancaster et al (Nature, 2013)

What is the relevance?

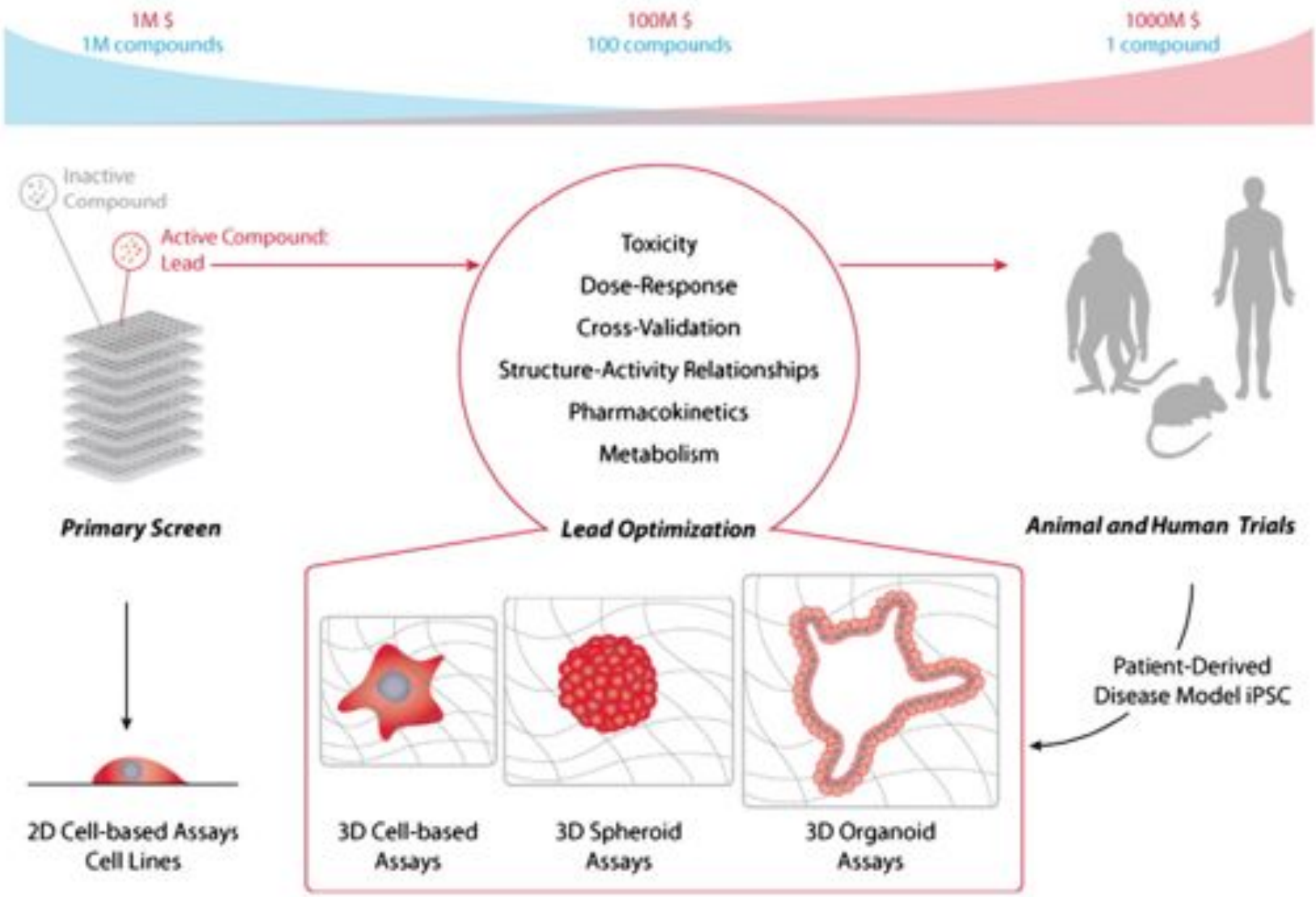
Organoid-based therapy



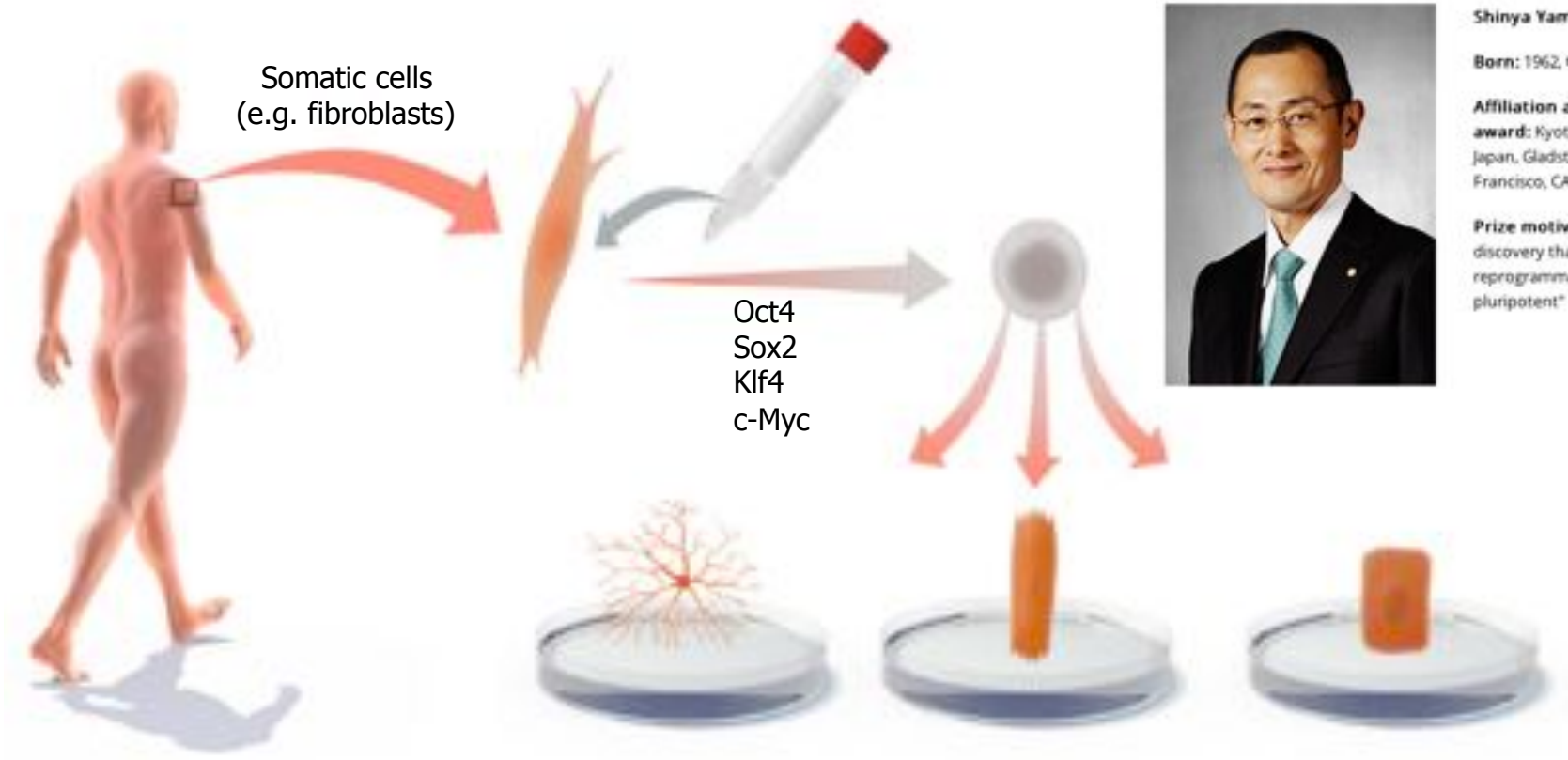


Yui et al (Nat Med, 2012)

Drug discovery



Induced pluripotent stem (iPS) cells



The Nobel Prize in Physiology or Medicine 2012
Sir John B. Gurdon, Shinya Yamanaka

Shinya Yamanaka - Facts

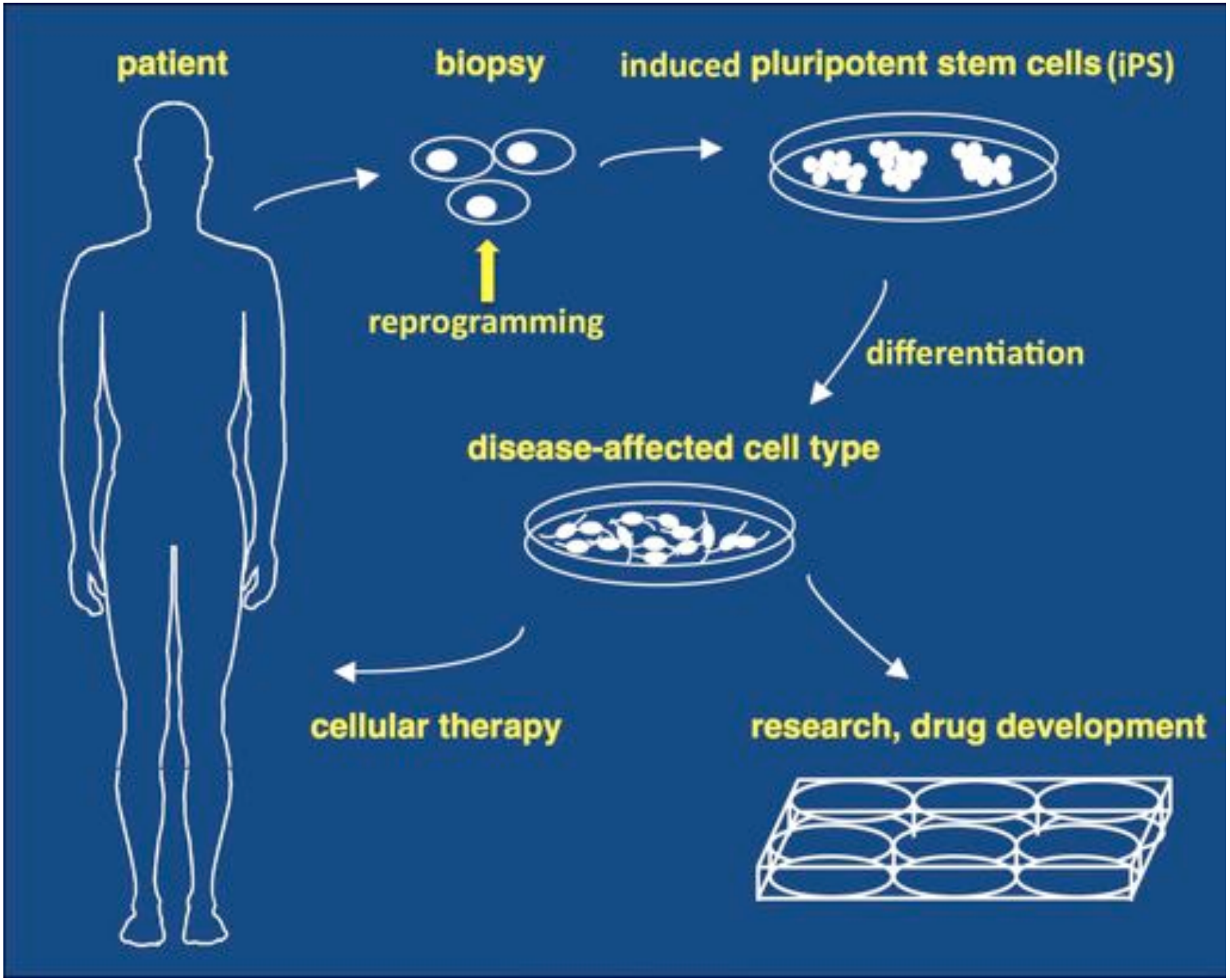
Shinya Yamanaka

Born: 1962, Osaka, Japan

Affiliation at the time of the award: Kyoto University, Kyoto, Japan, Gladstone Institutes, San Francisco, CA, USA

Prize motivation: "for the discovery that mature cells can be reprogrammed to become pluripotent"

http://www.nobelprize.org/nobel_prizes/medicine/laureates/2012/popular-medicineprize2012.pdf


patient
biopsy
induced pluripotent stem cells (iPS)
reprogramming
differentiation
disease-affected cell type
cellular therapy
research, drug development

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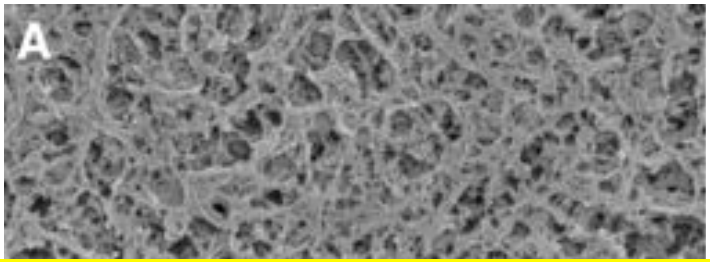
Bioengineering challenges

1. 3D matrix engineering to optimize stem cell-based organogenesis

2. Patterning organ formation

3. Organ-on-a-chip => probing function

3D culture: more sophisticated, but undefined



ECM Composition of BD Matrigel Matrix vs. GFR

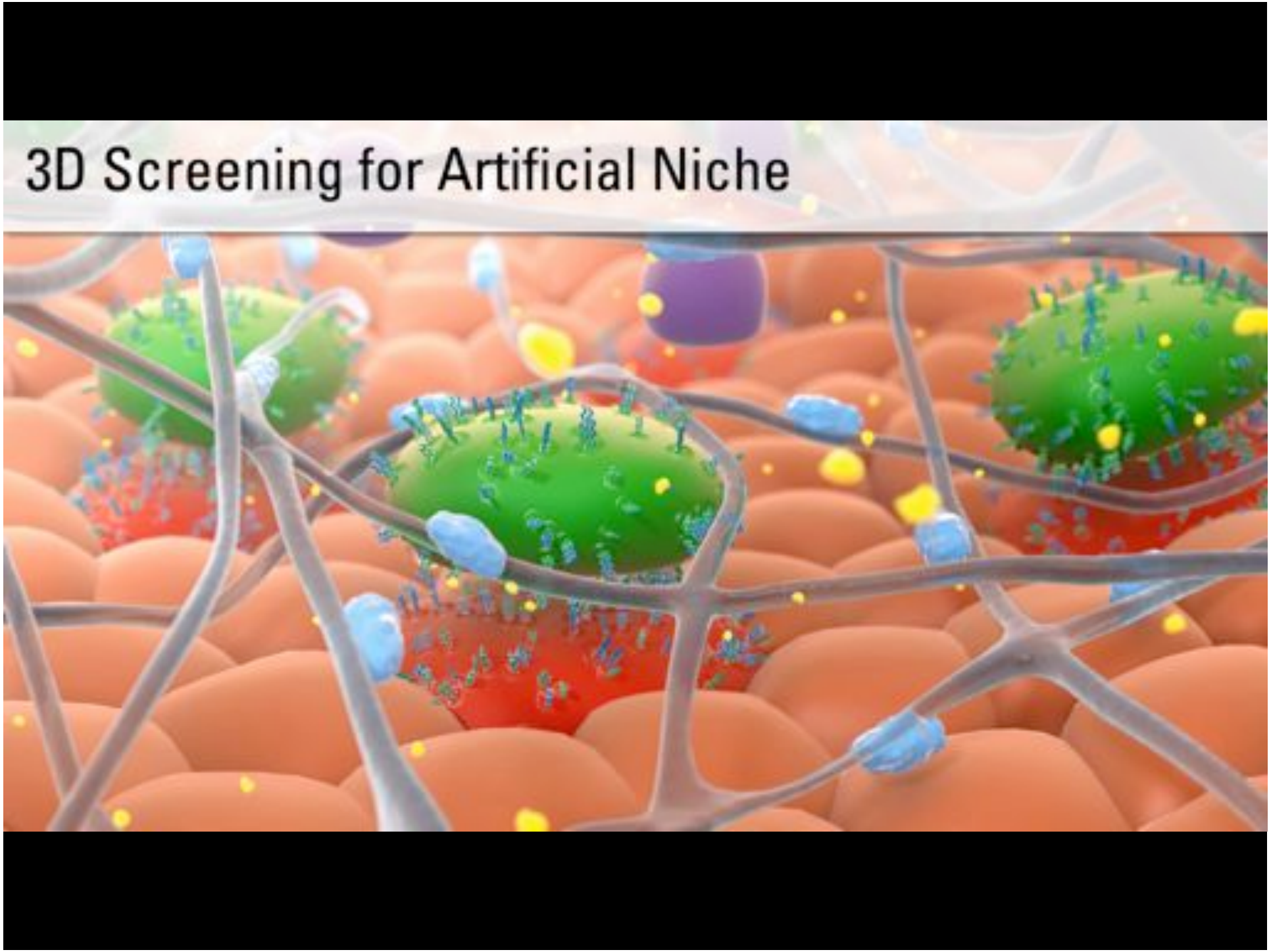
BD Matrigel Matrix Component	Percent in BD Matrigel Matrix	Percent in BD Matrigel Matrix GFR
Laminin	56%	61%
Collagen IV	31%	30%
Entactin	8%	7%

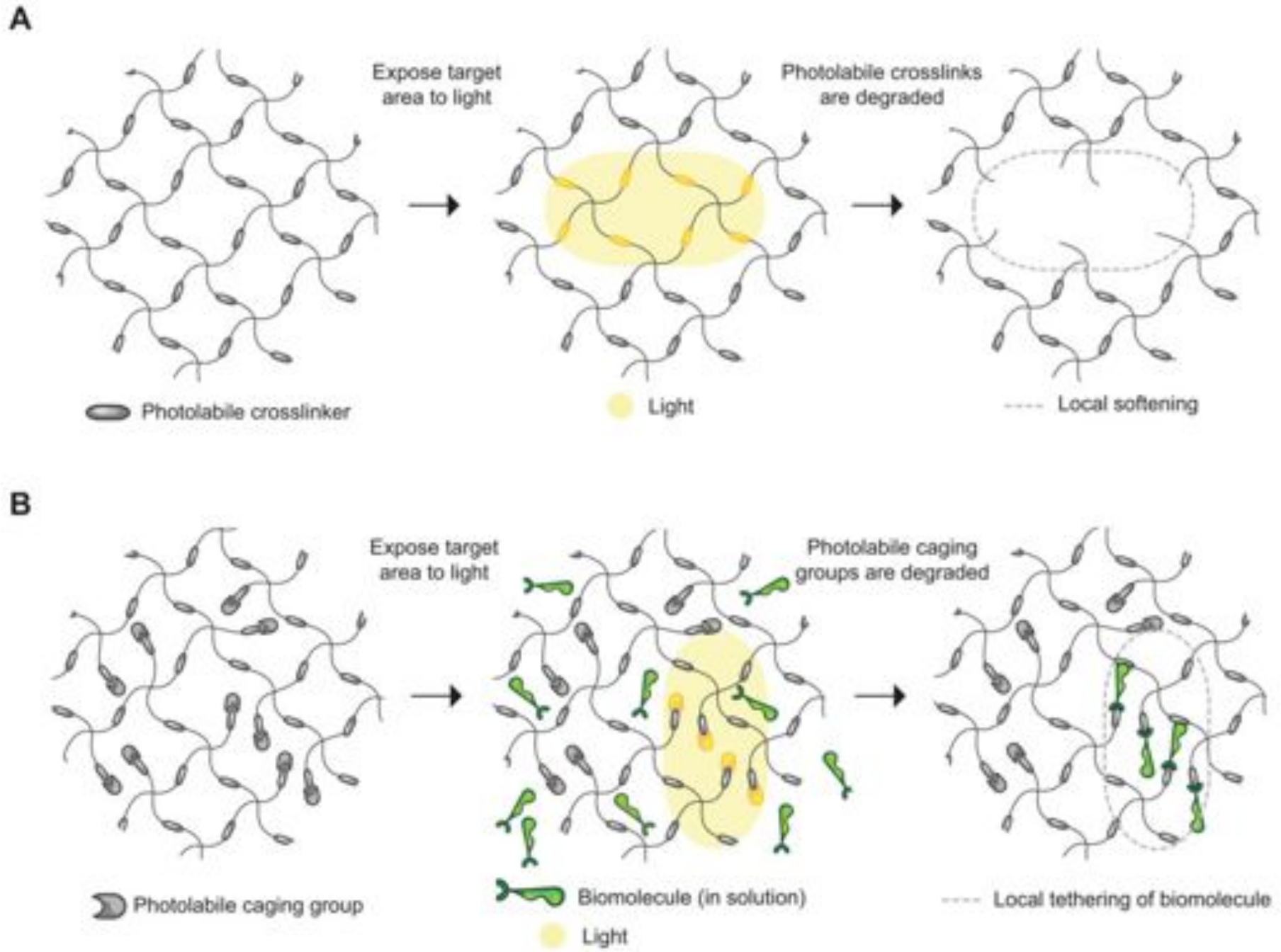
Average Growth Factor Concentrations in BD Matrigel Matrix

Parameter	BD Matrigel Matrix	BD Matrigel Matrix GFR
bFGF (pg/ml)	0 - 0.1	n.d.
EGF (ng/ml)	0.5 - 1.3	< 0.5
IGF-1 (ng/ml)	15.6	5
PDGF (pg/ml)	12	< 5

Limitations:

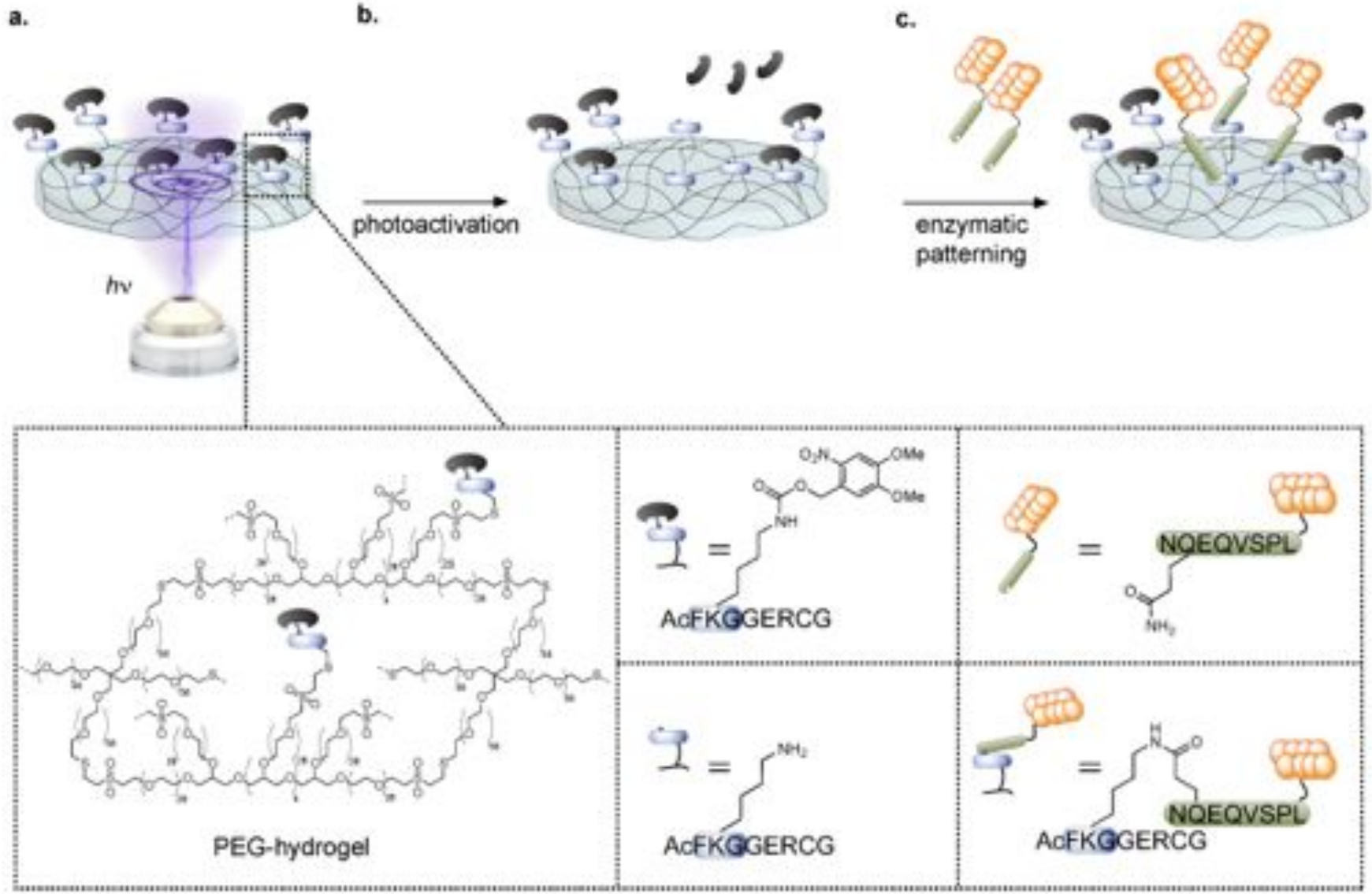
*** Fixed composition => cell-, tissue- and cell fate-specificity?**
*** Batch-to-batch variability => reproducibility?**
*** Poor handling properties => automation? miniaturization?**
*** Animal ECM-derived => clinical relevance?**


3D Screening for Artificial Niche



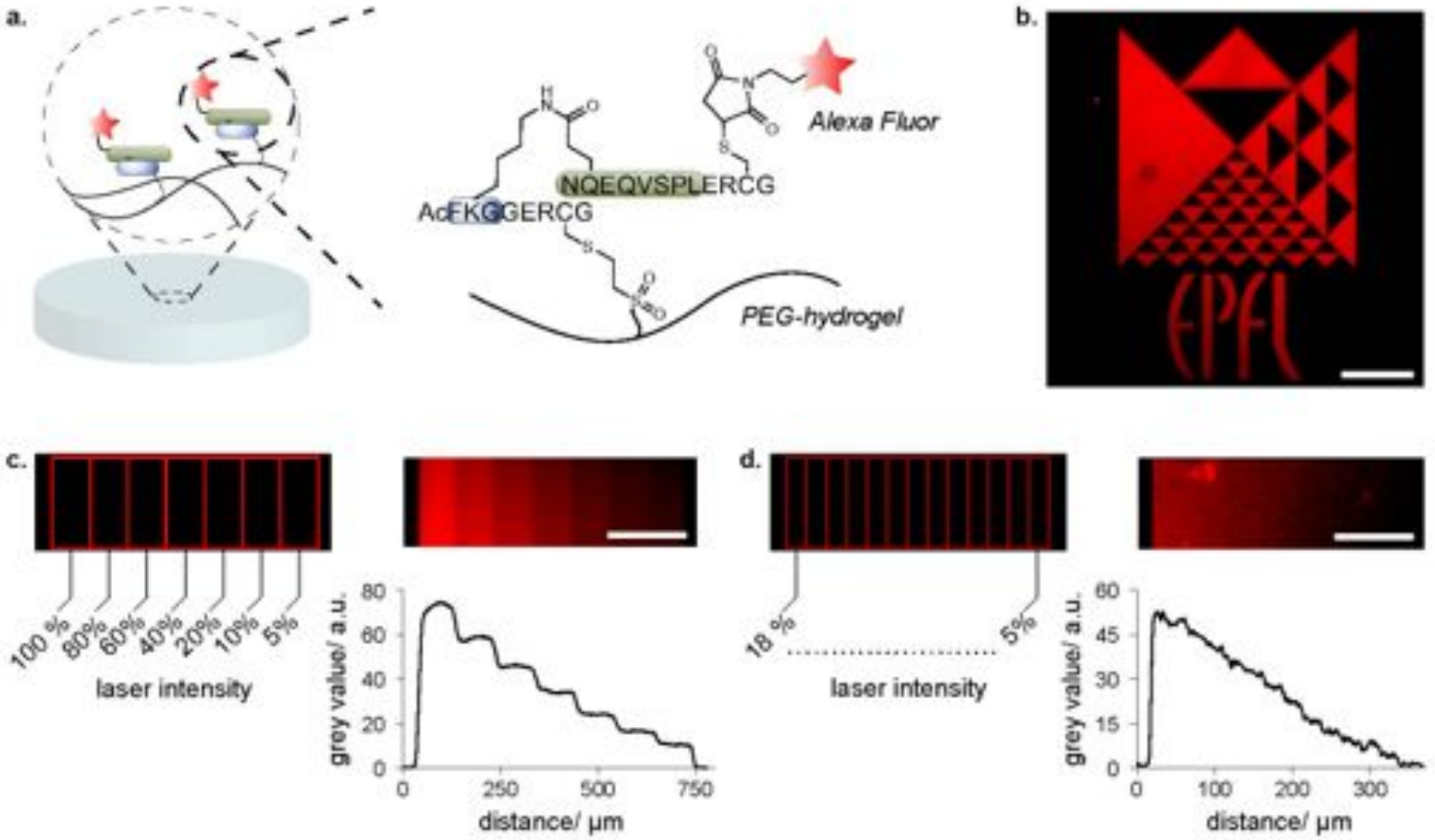
Gjorevski et al., Development, 2014

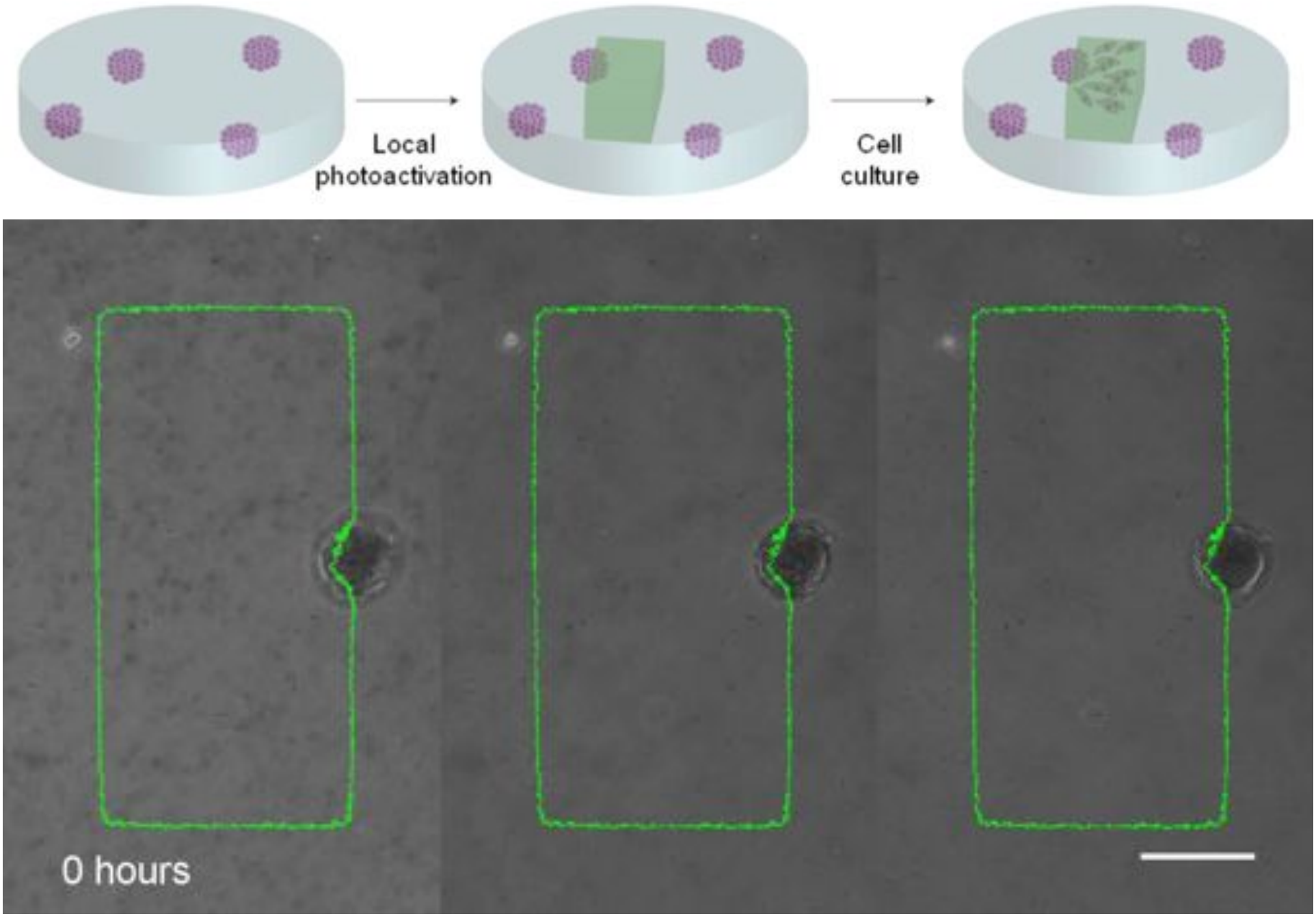
‘Photocaged’ hydrogel matrices: Light-controlled localized patterning of biomolecules

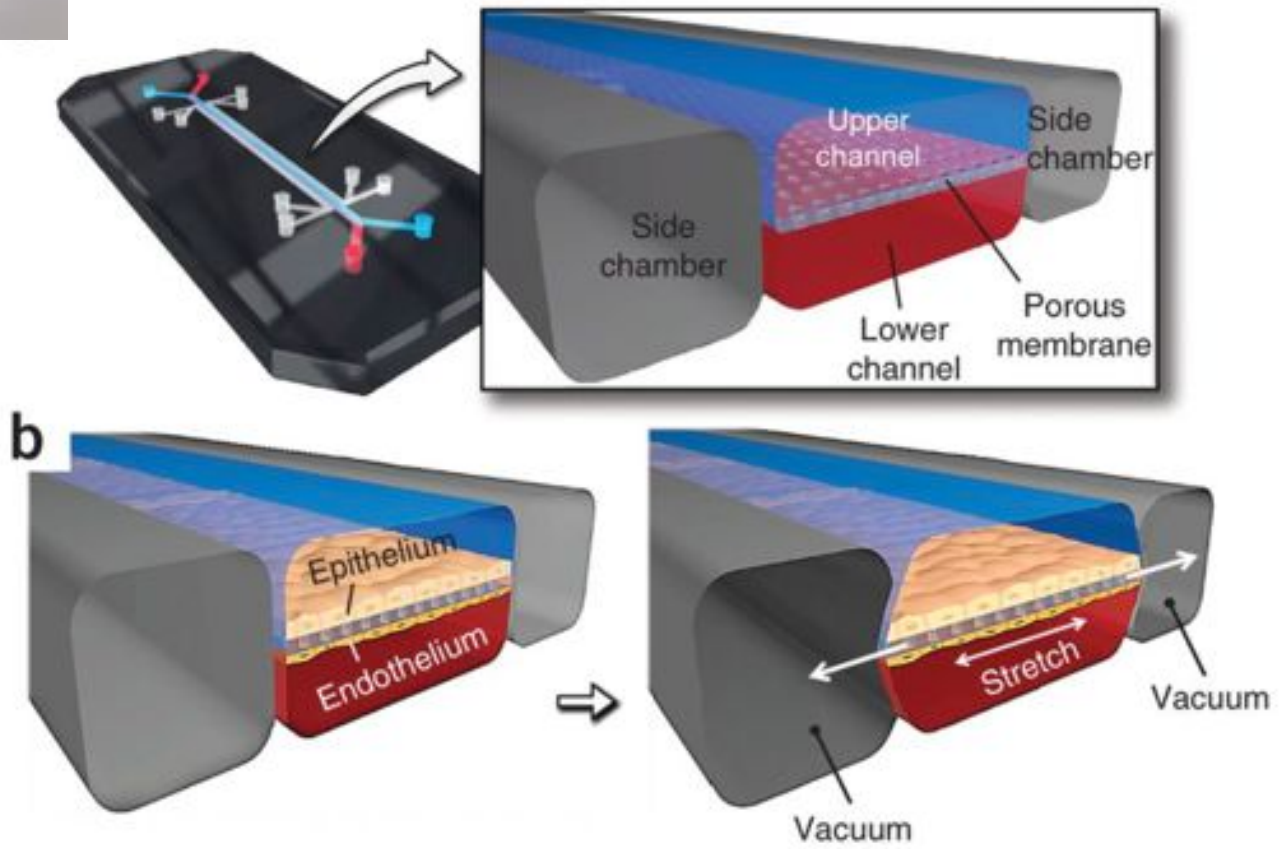


Kolb and Mosiewicz et al., Nature Materials, 2013

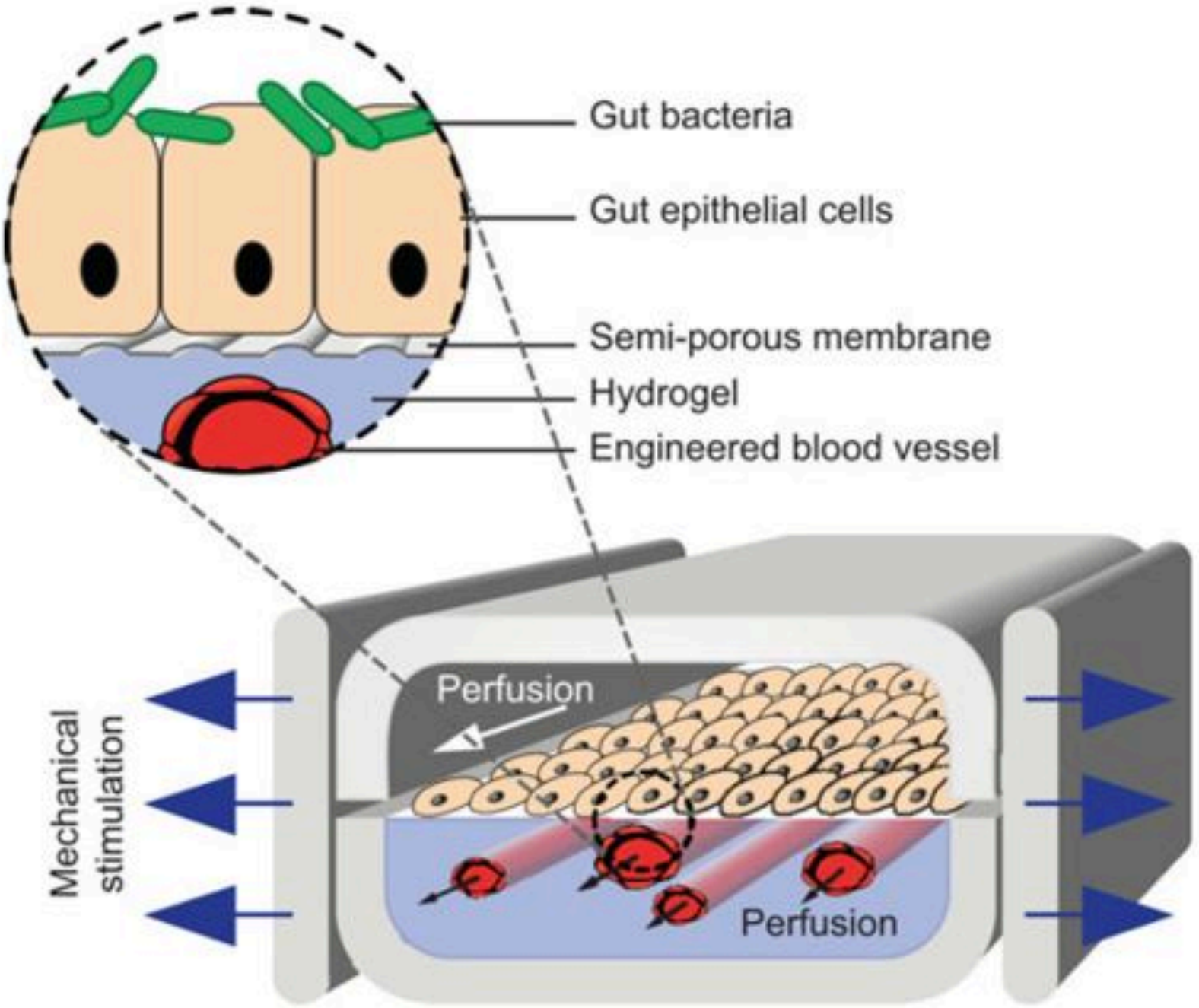
Virtually any pattern and any signal possible




Local
photoactivation
Cell
culture
0 hours



Huh et al, Nat Protoc, 2013



Gjorevski et al., Development, 2014

Overview

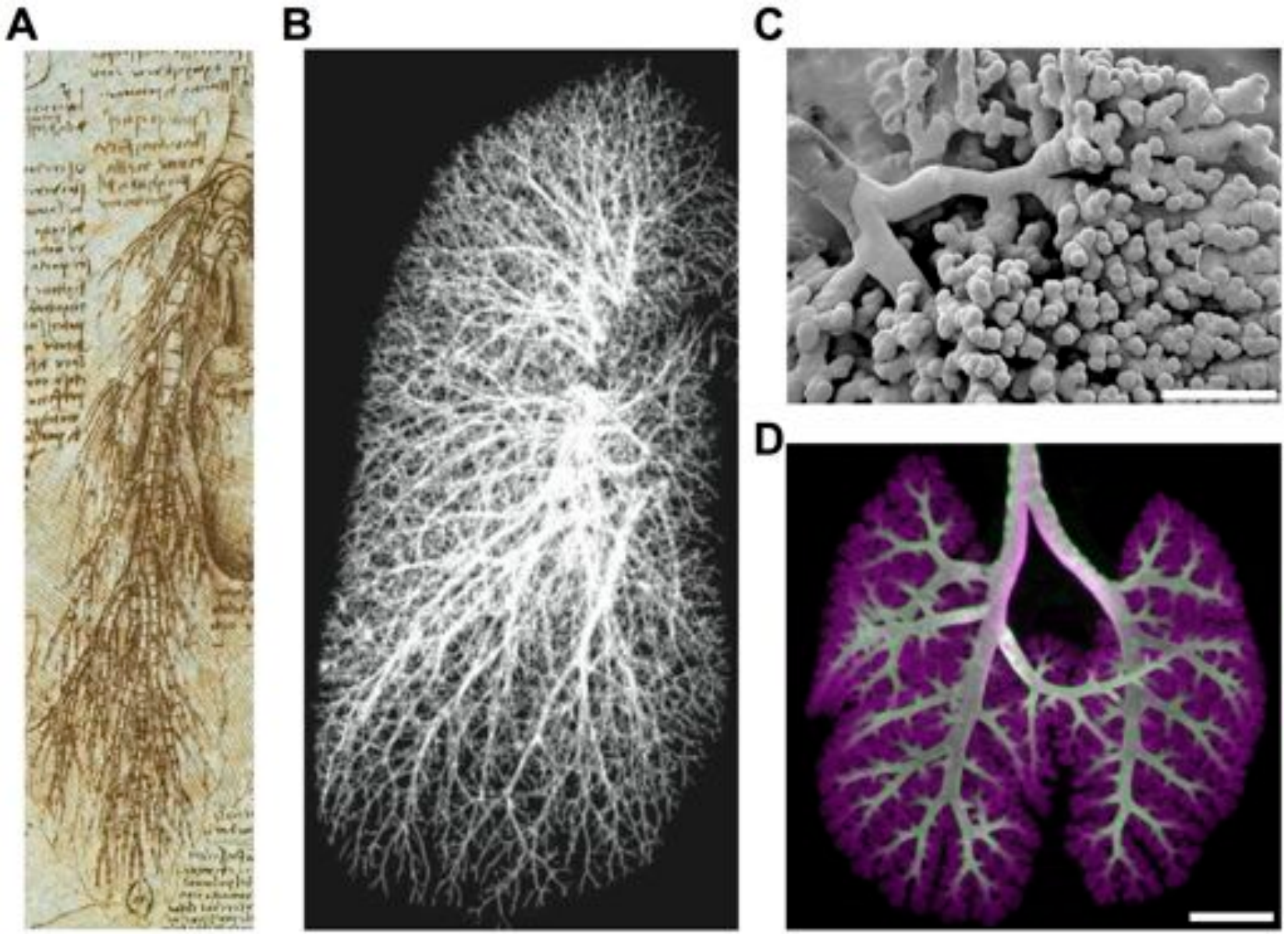
1. Tissue Engineering 1.0: the beginning

2. *Ex situ* versus *in situ* tissue engineering

3. Stem cell-based organogenesis and its applications

4. Bioengineering tricks to guide and exploit organogenesis

5. Bioprinting and vascularization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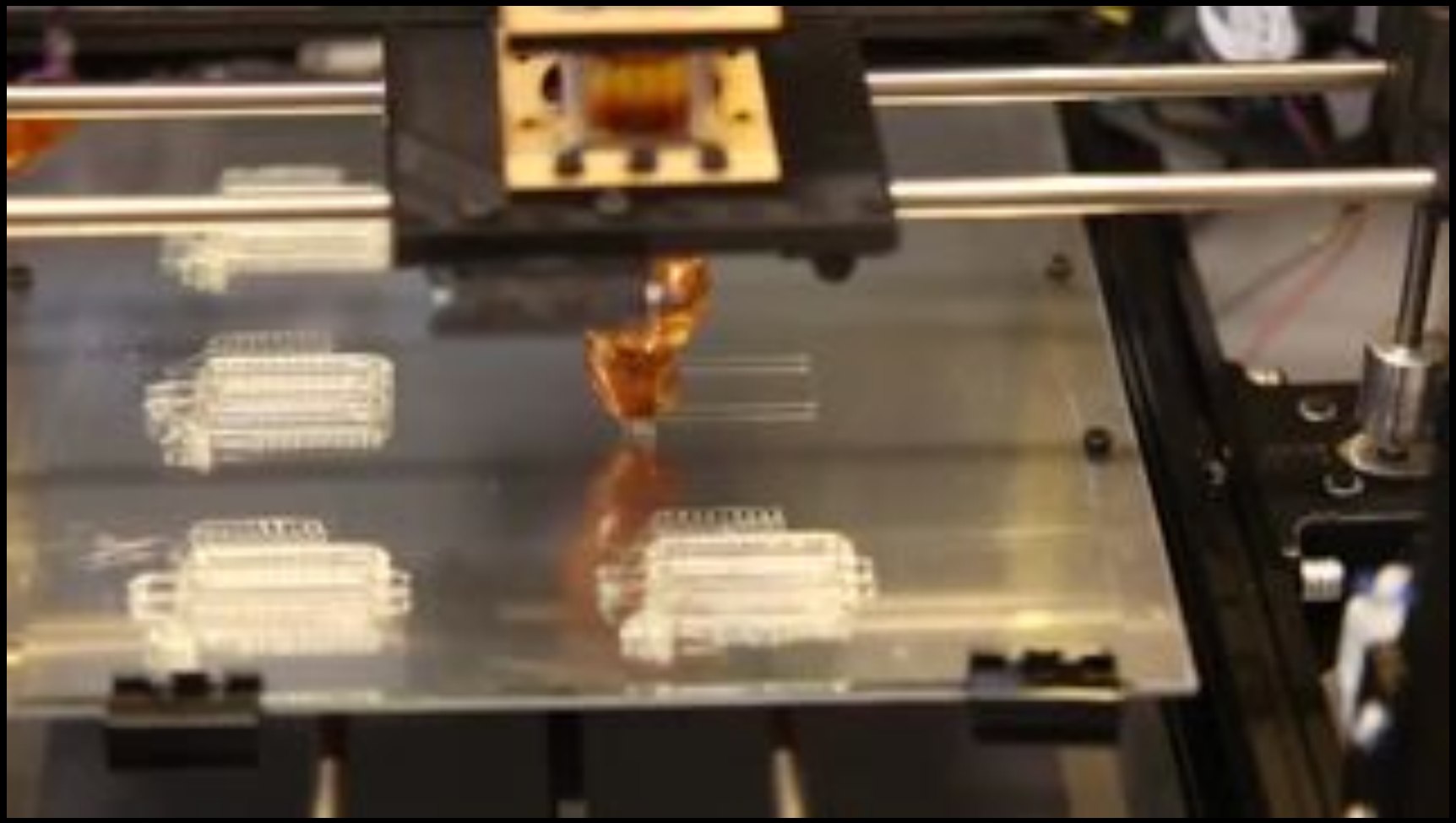

Jordan Miller, Chris Chen, Sangeeta Bhatia

Not quite there yet…

Questions?