SYSFATE

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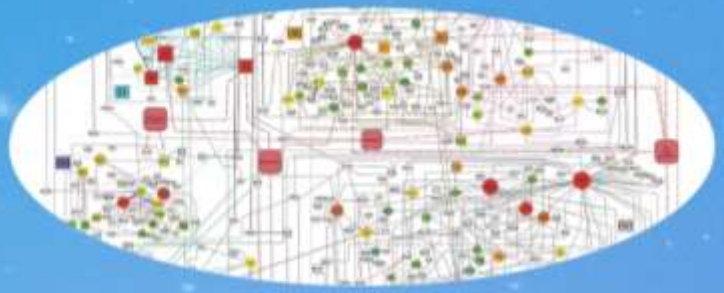
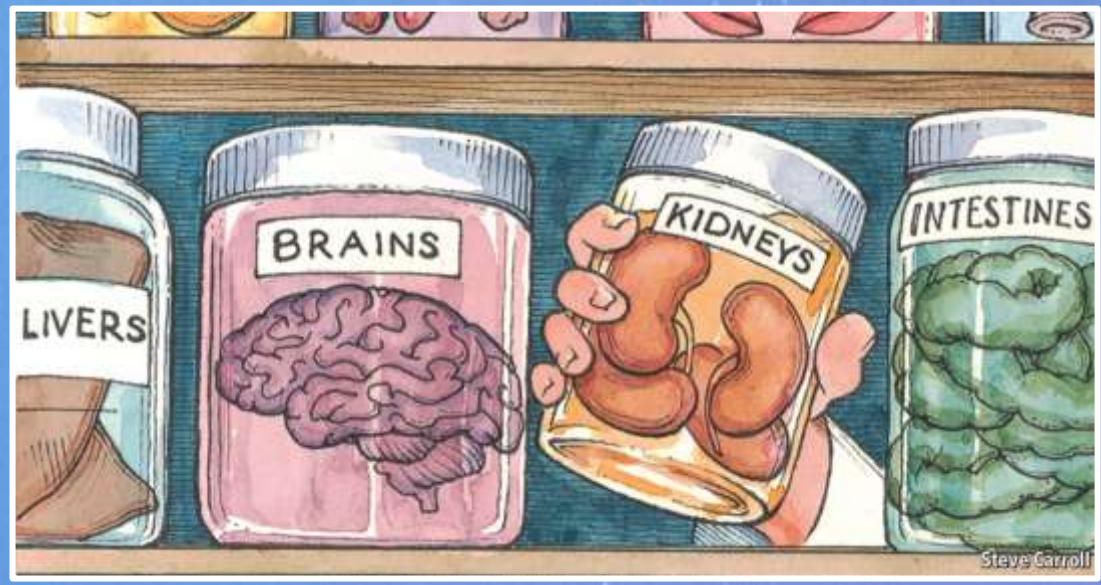
SysFate

Systems Biology of cell Fate decisions

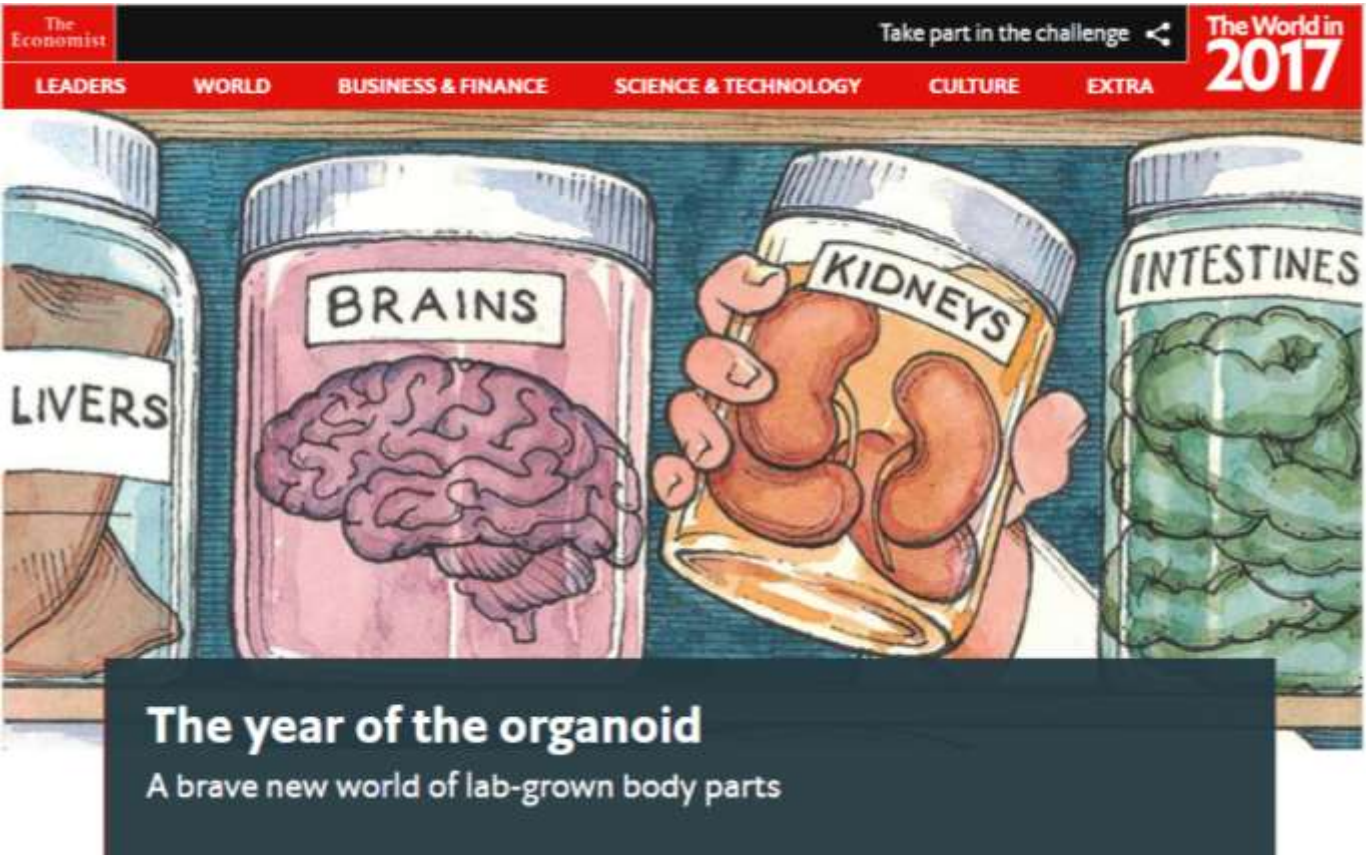


Marco Antonio Mendoza
Laboratory of Systems Biology

Organoids as an alternative for animal models



ISSB laboratory; UMR 8030
Génomique Métabolique



Geoffrey Carr

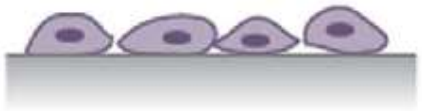
SCIENCE AND TECHNOLOGY

The brain-in-a-jar is one of science fiction's creepier ideas. It is a safe prediction that 2016 will not see such literally disembodied people become reality. What it will see, though, is the blossoming of a ***technology that lets scientists grow things resembling brains—and also livers, kidneys, intestines and many other body parts—in glass vessels in laboratories***.

Over the course of ***2016 these simulacra***, ***known as organoids***, ***will begin to enter routine medical use as ways of testing drugs***. ***They will also, by illuminating the way real organs grow, cast light on diseases caused during embryonic development.*** Eventually, though probably not in 2016, ***some will even be made good enough to be transplanted into people, to replace diseased or failing natural organs.***

….

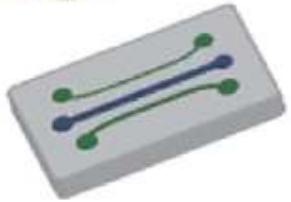
Biological Model Systems



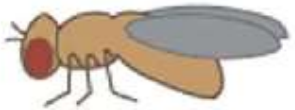
2D cell culture

organoids

3D cell culture



Organoids
Organ-on-a-chip



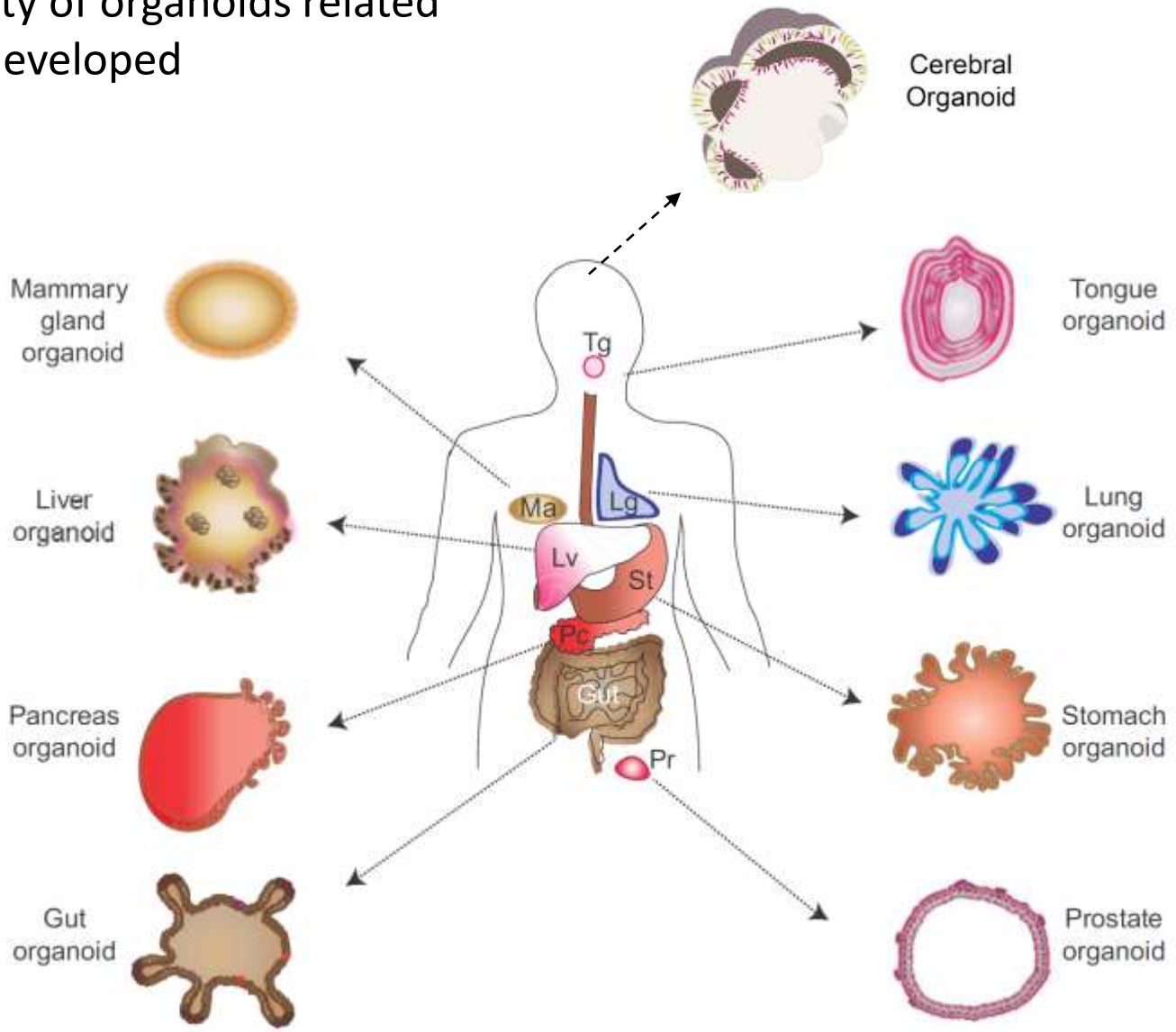
Model organisms

Humans

Experimental Tractability

Physiological Relevance

Over the last years a variety of organoids related to different organs were developed



What is an organoid?

- three-dimensional structure
- self-organized
- Reconstitutes (at least partially) tissue architecture (3D) and functions of the related organ

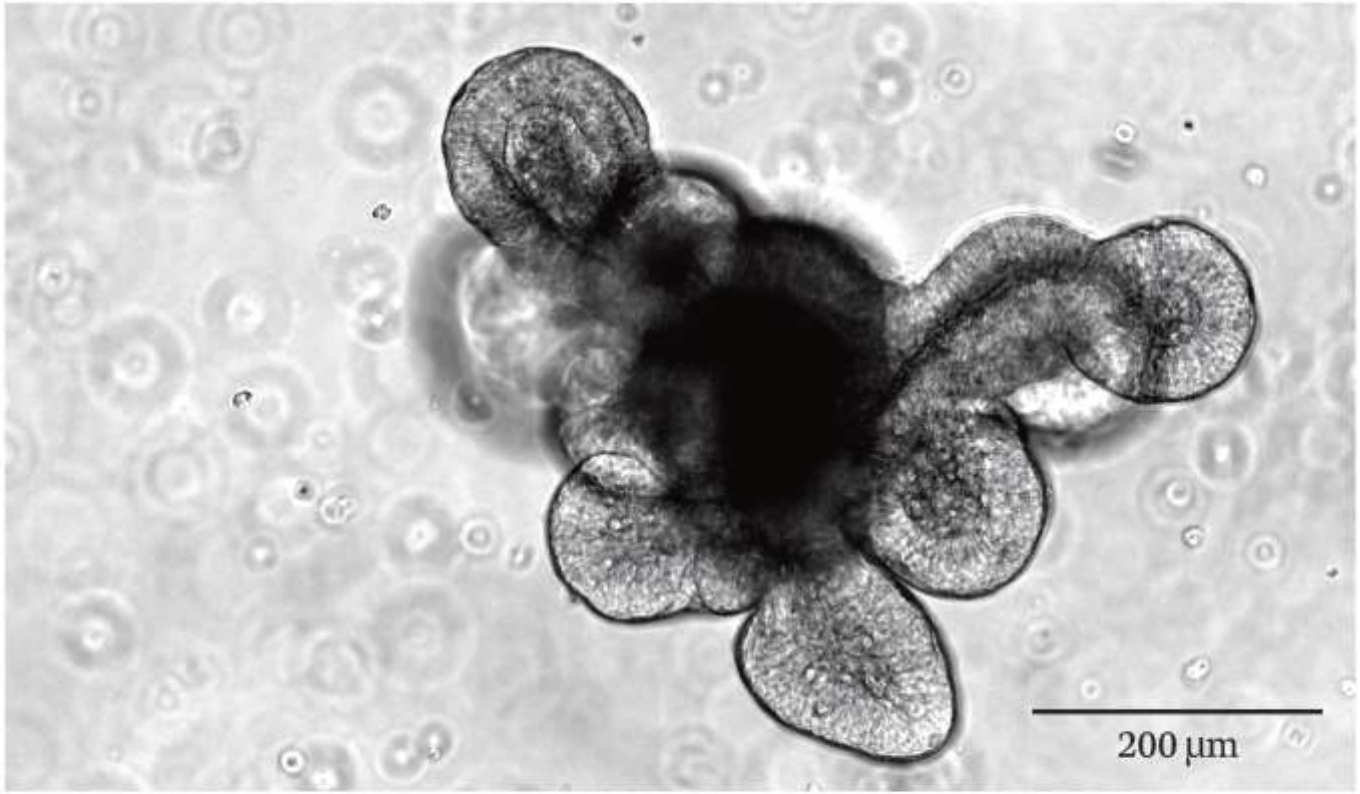


Figure 1. Light microscope visualization of a mouse intestinal organoid.

Source: STEMCELL Technologies

Other in-vitro strategies sharing characteristics of organoids

organoid

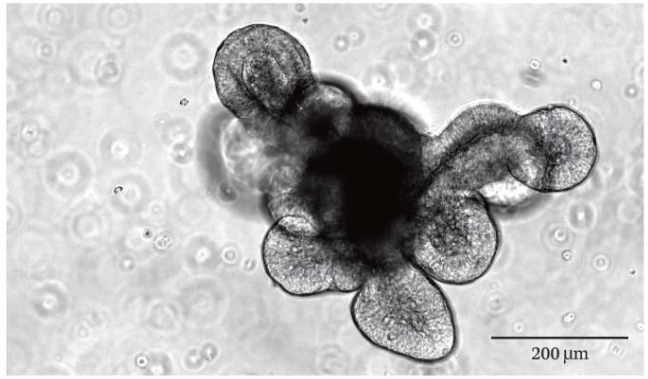
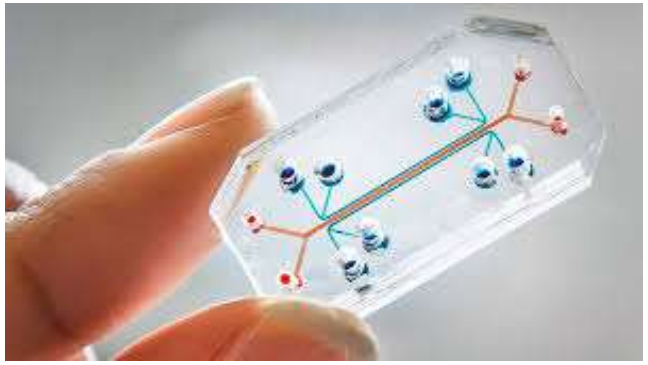
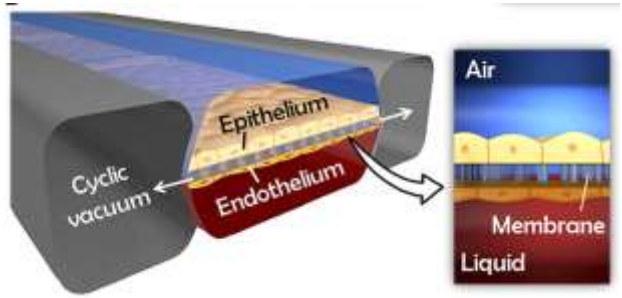


Figure 1. Light microscope visualization of a mouse intestinal organoid.
Source: STEMCELL Technologies

- three-dimensional structure
- self-organized
- Reconstitutes (at least partially) tissue architecture (3D) and functions of the related organ

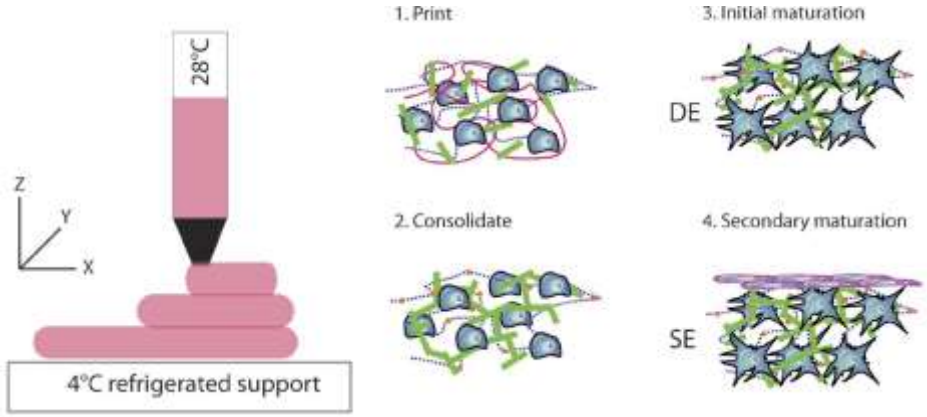
Organs on chip

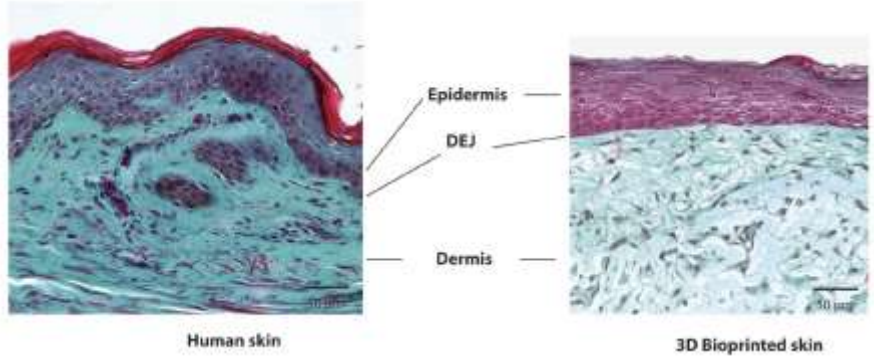




multi-channel 3-D microfluidic cell culture chip that ***simulates the activities, mechanics and physiological response*** of entire organs and organ systems.

3d-bioprinted tissues

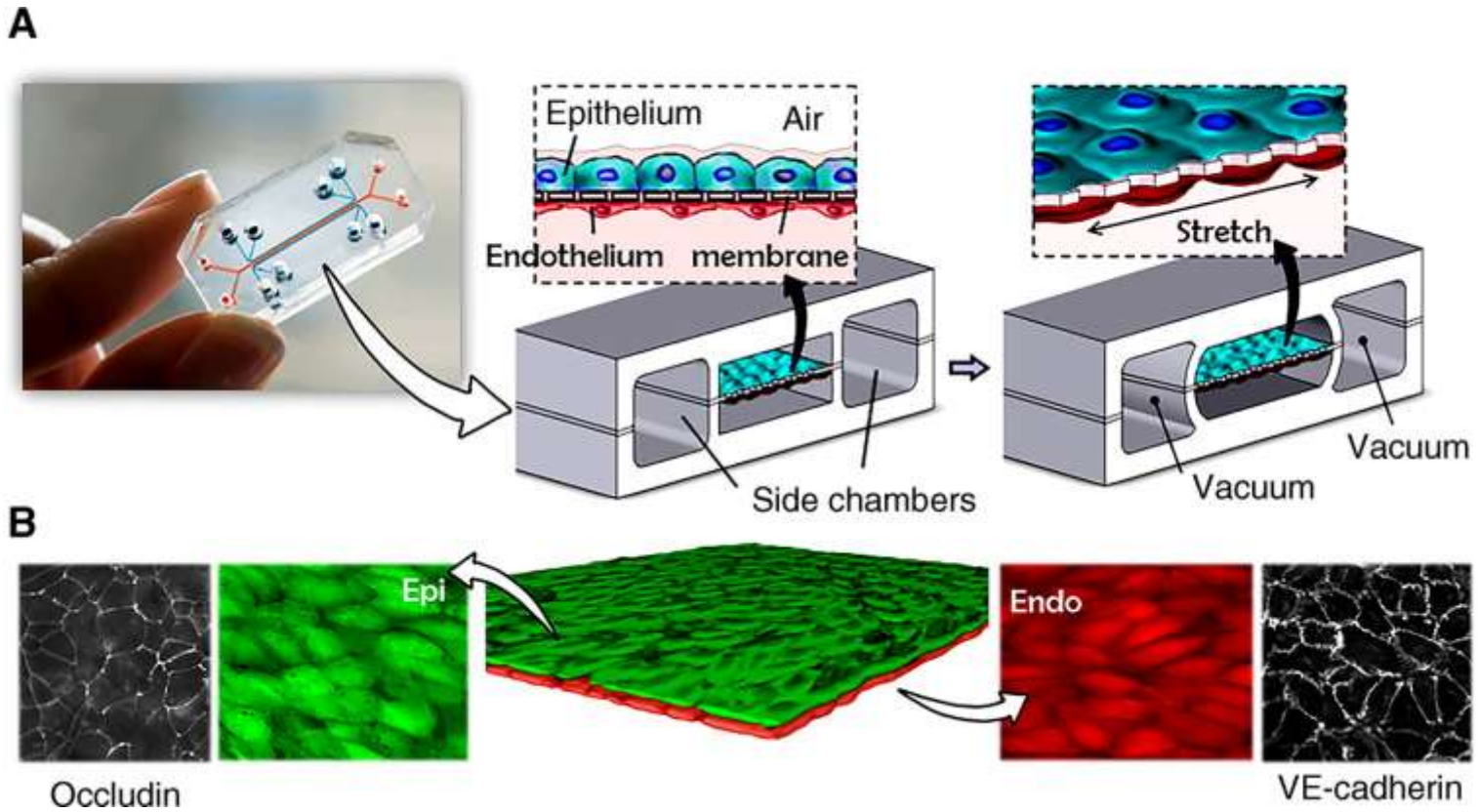




in-vitro ***reconstruction of complex tissue architecture*** by systematic cells deposition (bio-ink).

Organs on chip:

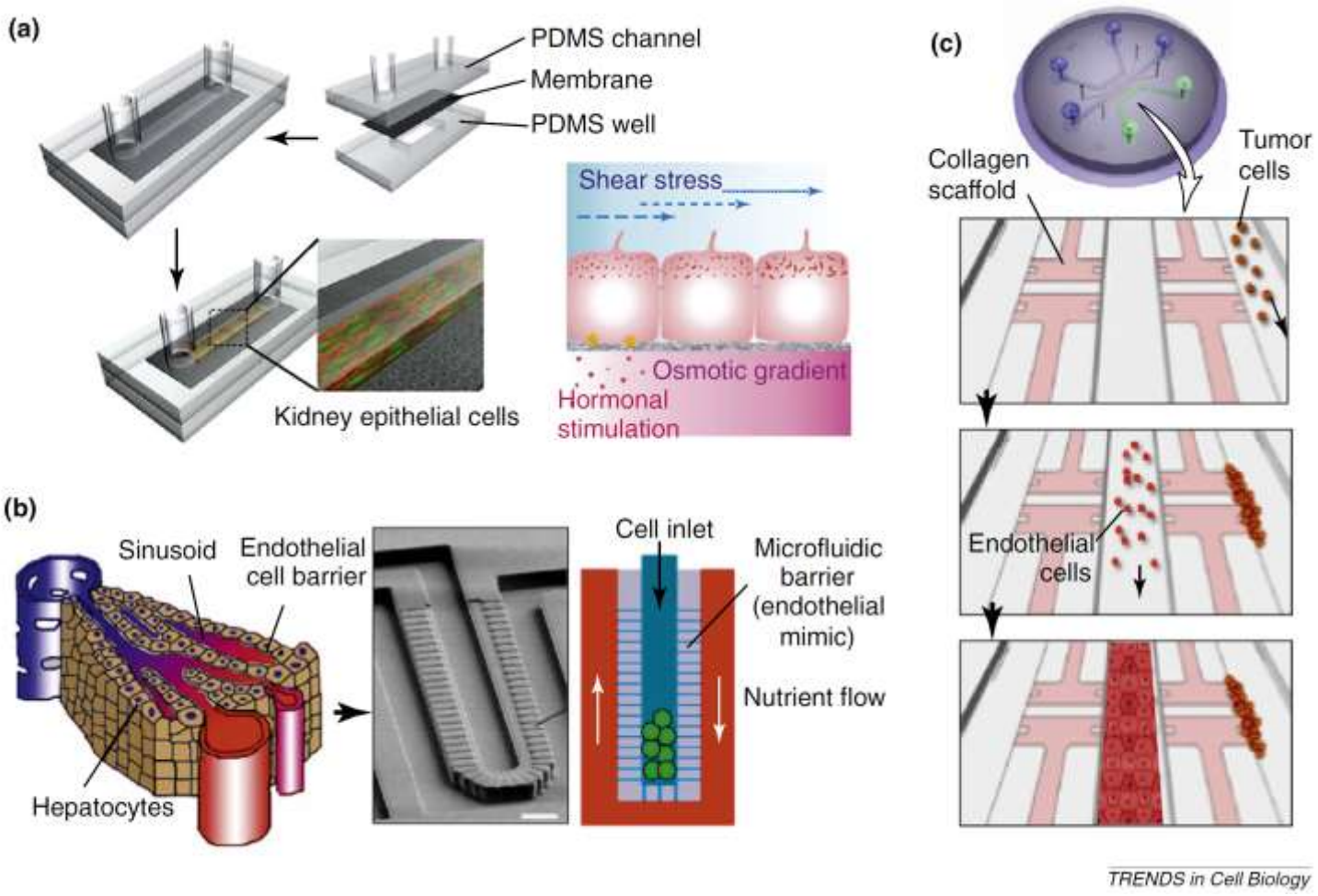
A human breathing lung-on-a-chip (*Huh DD et al; Ann Am Thorac Soc.* ***2015***)



Differentiated cells are deposited on microfabricated chambers, as a way to recreate physiological events in-vitro

Figure 1. A human breathing lung-on-a-chip. (A) The microfabricated lung mimic device recreates physiological breathing movements by applying vacuum to the side chambers and causing mechanical stretching of the poly(dimethylsiloxane) membrane forming the alveolar–capillary barrier. (B) Longterm microfluidic co-culture produces a tissue–tissue interface consisting of a single layer of the alveolar epithelium (Epi; green) closely apposed to a monolayer of the microvascular endothelium (Endo; red), both of which express intercellular junctional structures such as occludin or vascular endothelial (VE)-cadherin.

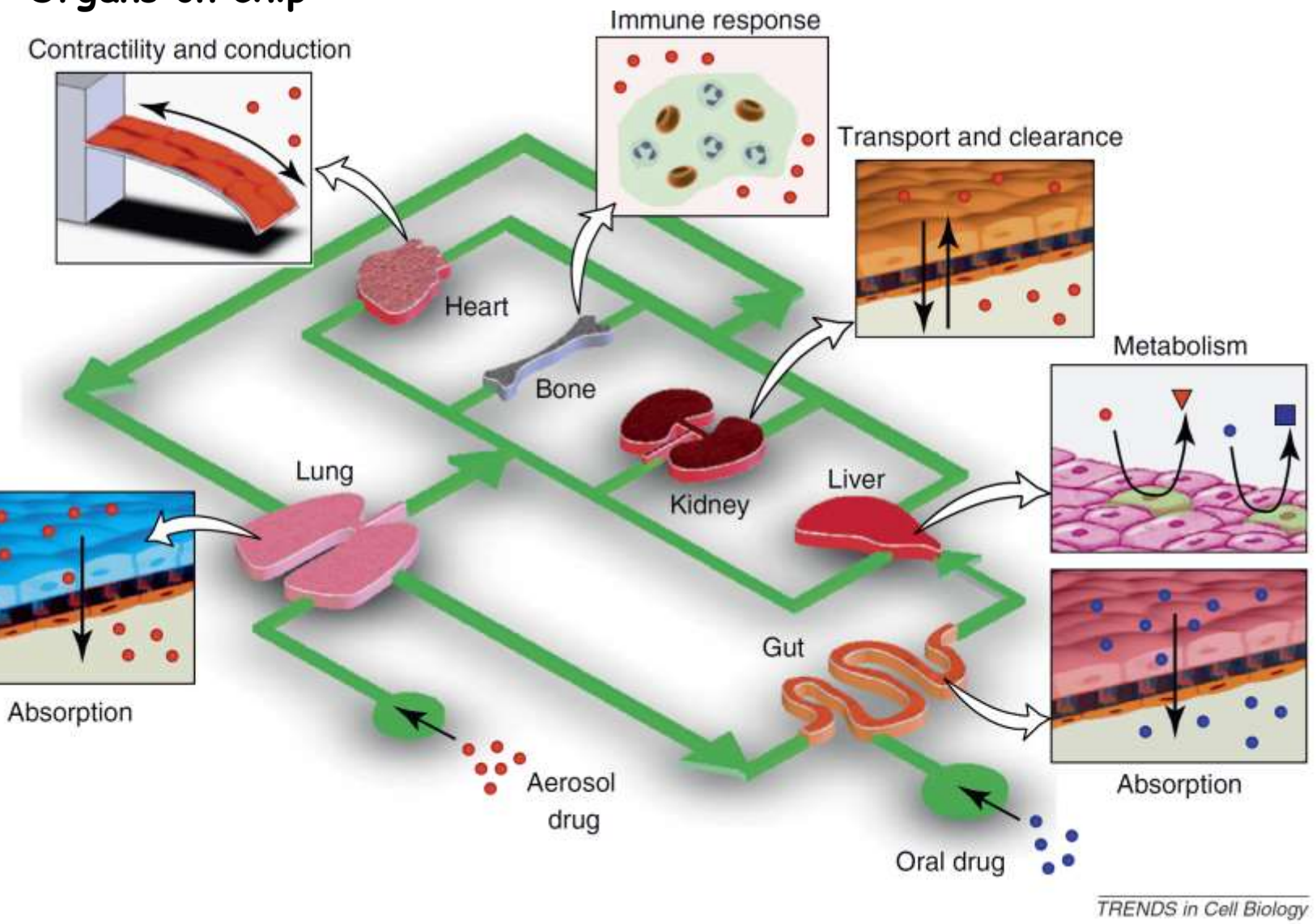
Organs on chip:



(a) A **microfluidic kidney epithelium model** composed of a multilayered microdevice that incorporates stacked layers of PDMS microchannels and a PDMS well separated by a porous polyester membrane. The 3D architecture of this microsystem provides physiologically relevant culture environments for polarized kidney epithelial cells, and enables precise control of fluid flows, selective exposure of the apical and basal sides of the cells to fluid shear, hormones, and chemical gradients, and collection of samples from both sides of the polarized tissue. **(b) A microengineered liver-on-a-chip reconstitutes hepatic microarchitecture**. The functional unit of this microsystem consists of a central liver-cell culture chamber and a surrounding nutrient flow channel separated by microfabricated barrier structures patterned with a set of narrow (2 mm in width) microchannels that mimic the highly permeable endothelial barrier between hepatocytes and the liver sinusoid. This biomimetic device closely approximates transport of nutrients and waste products in the liver sinusoid and provides more favorable environments for the maintenance of primary liver cells in a differentiated state (scale bar, 50 mm). **(c) Heterotypic interactions between tumor cells and endothelial cells** are studied in a microfluidic device that permits co-culture of these cells in two separate microchannels connected via scaffold channels filled with 3D collagen gels (pink channels in the diagram). This microfluidic system is used to model the tumor microenvironment and gain better understanding of important disease processes such as angiogenesis and cancer cell invasion during cancer progression.

Dongeun et al, Trends in Cell Biology 2011

Organs on chip:



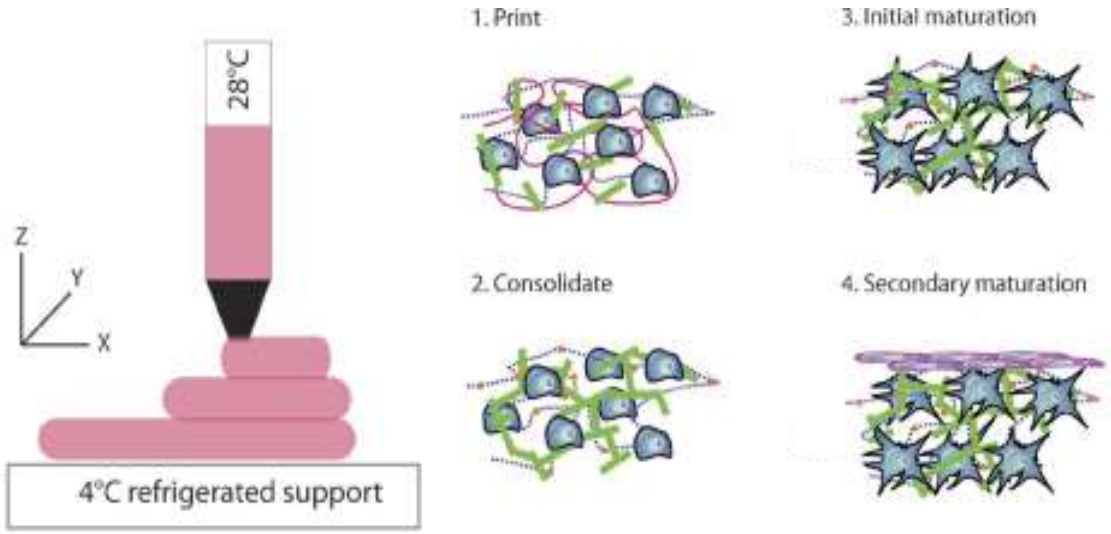
The human-on-a-chip concept. Biomimetic microsystems representing different organs can be integrated into a single microdevice and linked by a microfluidic circulatory system in a physiologically relevant manner to model a complex, dynamic process of drug absorption, distribution, metabolism and excretion, and to more reliably evaluate drug efficacy and toxicity. As shown in this example, an integrated system of microengineered organ mimics (lung, heart, gut, liver, kidney and bone) can be used to study the absorption of inhaled aerosol drugs (red) from the lung to microcirculation, as well as to measure their cardiotoxicity (e.g. changes in heart contractility or conduction), transport and clearance in the kidney, metabolism in the liver, and immune-cell contributions to these responses. Drug substances (blue) also can be introduced into the gut compartment to investigate interplay between orally administered drugs and molecular transporters and metabolizing enzymes expressed in the various organs.

Dongeun et al, Trends in Cell Biology 2011

3d-bioprinted tissues:

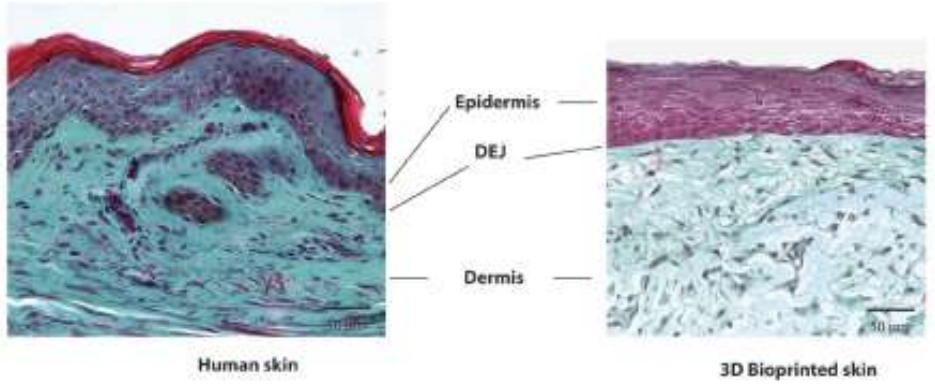
Human Skin 3D Bioprinting Using Scaffold-Free Approach

Léa J. Pourchet et al; Adv. Healthcare Mater. **2017**

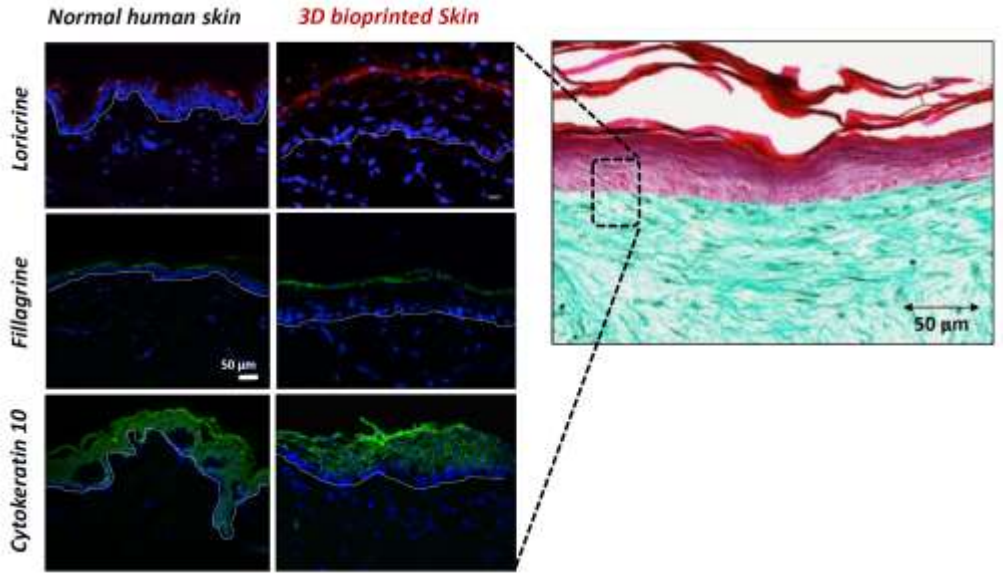


Schematic representation of the 3D bioprinting, consolidation, and maturation steps using the developed bio-ink.

The process necessary to the achievement of fully organized and functional 3D bioprinted skin was divided in three steps: the printing process itself, the consolidation in which gelatin was removed while alginate and fibrinogen were cross-linked, and the maturation during which printed cells were free to interact together and with the surrounding fibrinogen matrix.



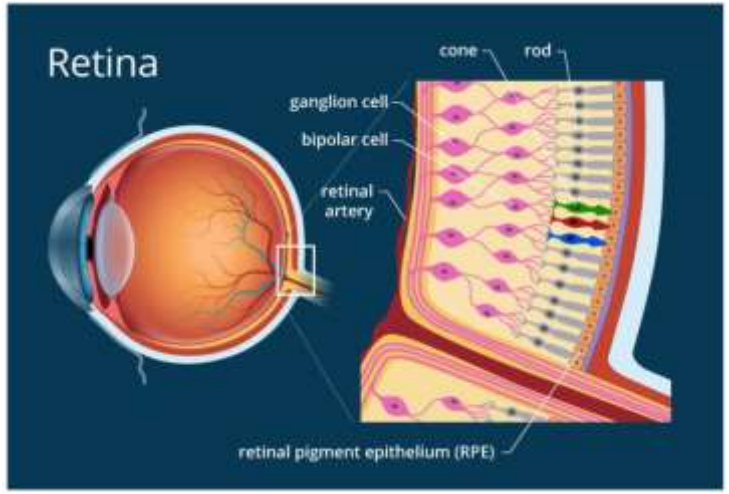
Histological and morphological characterization of the bioprinted skin. Optical microscopy images of normal human skin and bioprinted skin after 26 d of culture. Tissues were stained with Masson's Trichrome.



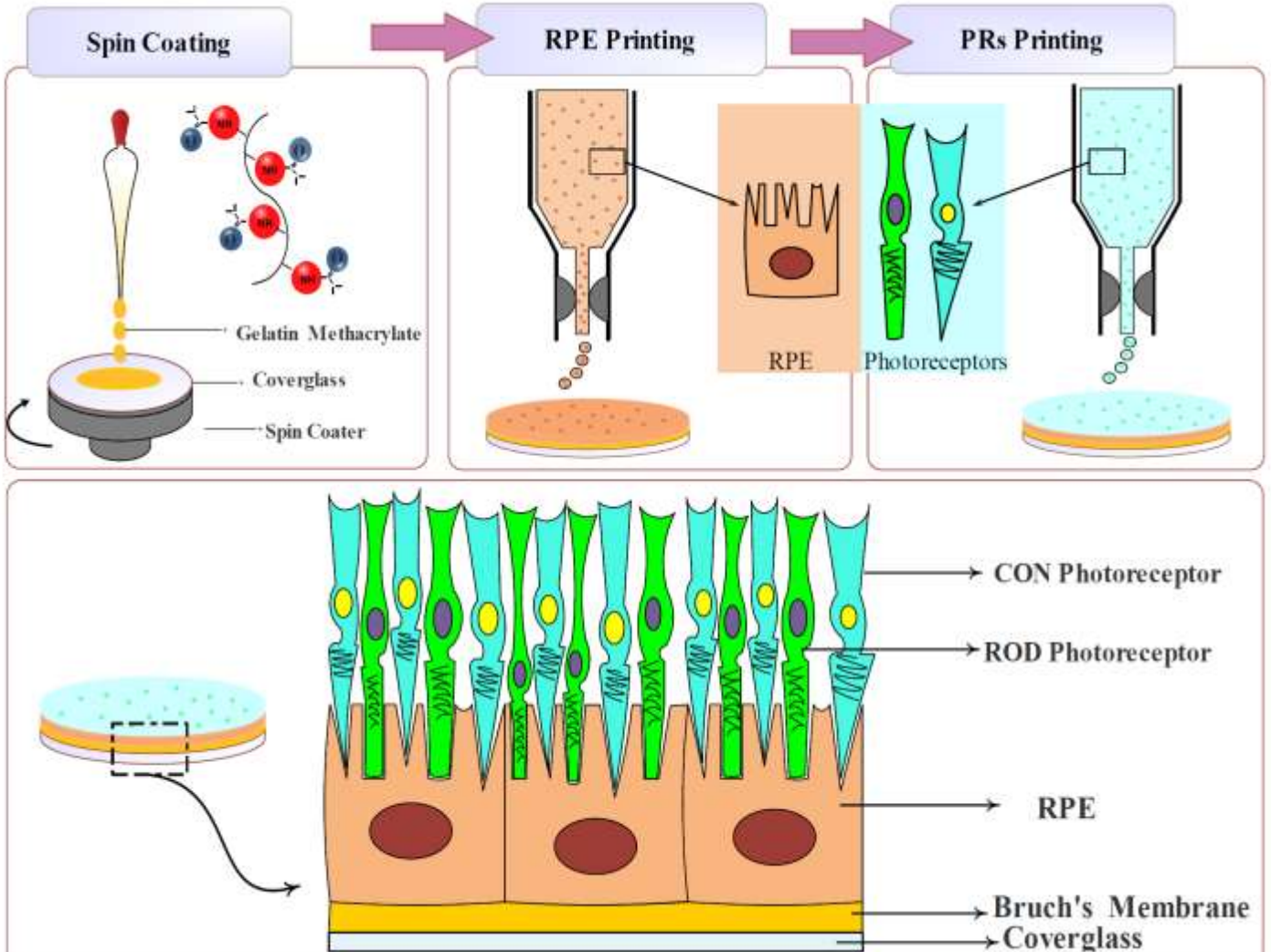
3d-bioprinted tissues:

Tissue engineering of retina through high resolution 3-dimentional inkjet bioprinting

Masaeli E et al; Biofabrication 2019



inkjet bioprinting system applied to the deposition of a photoreceptor cell (PRs) layer on top of a bioprinted retinal pigment epithelium (RPE), in a precise arrangement and without any carrier material.



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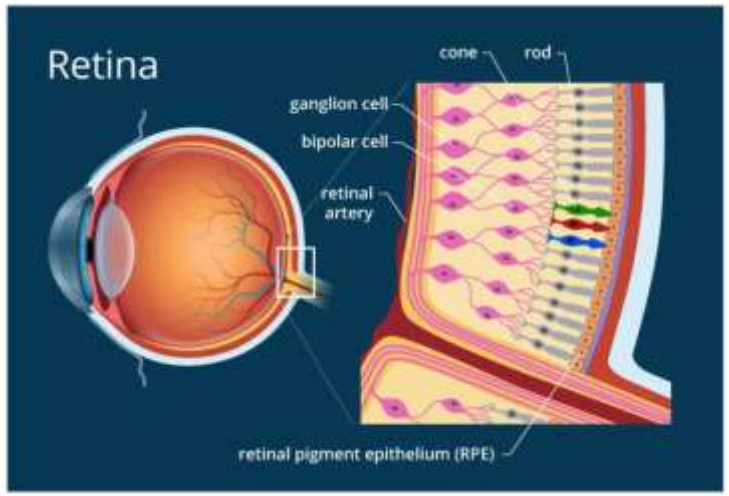
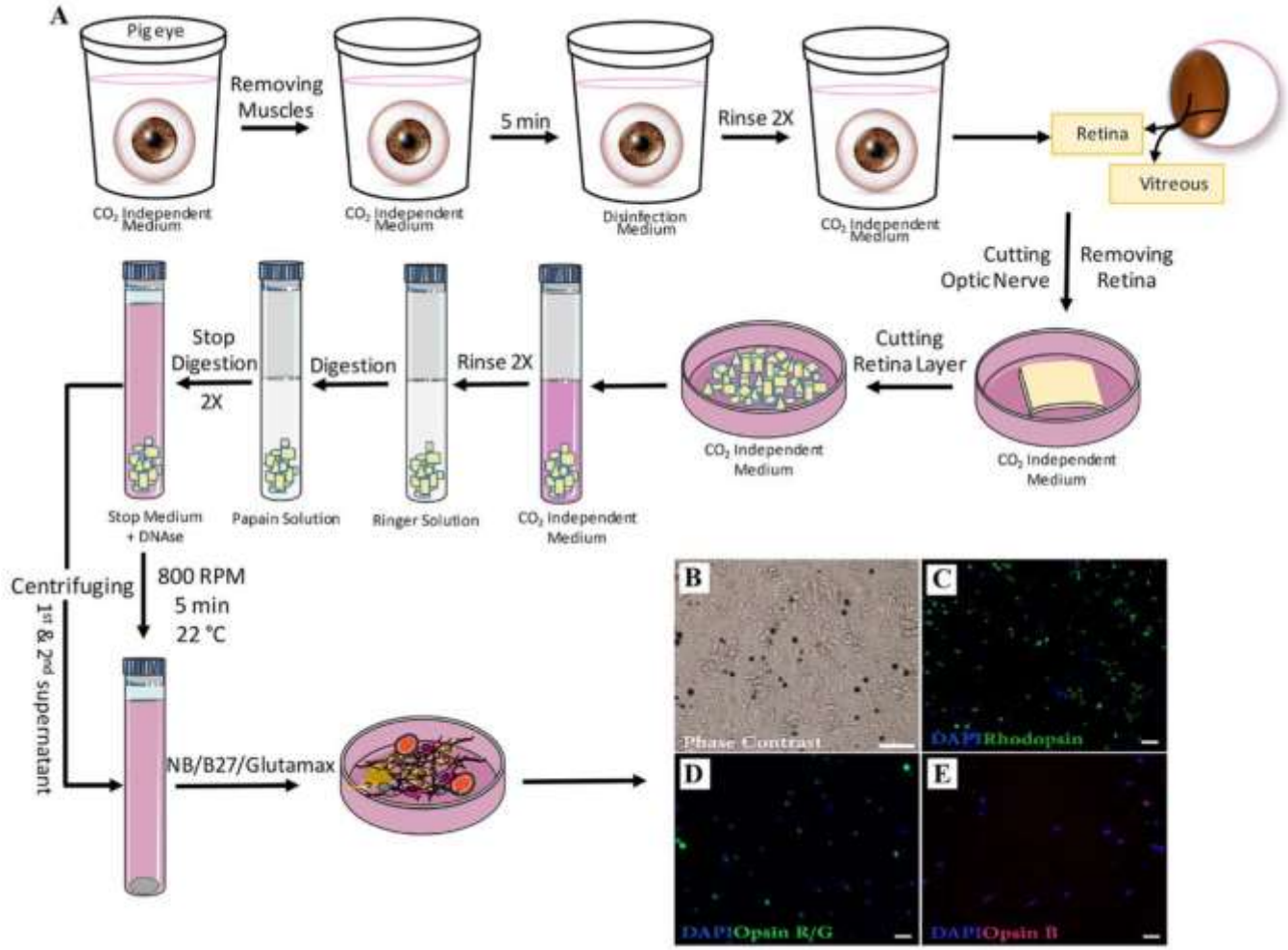


this carrier-free bioprinting method, allowed to develop a reasonable in vitro retina model for studding some sight-threatening diseases such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP).



Other in-vitro strategies sharing characteristics of organoids

organoid

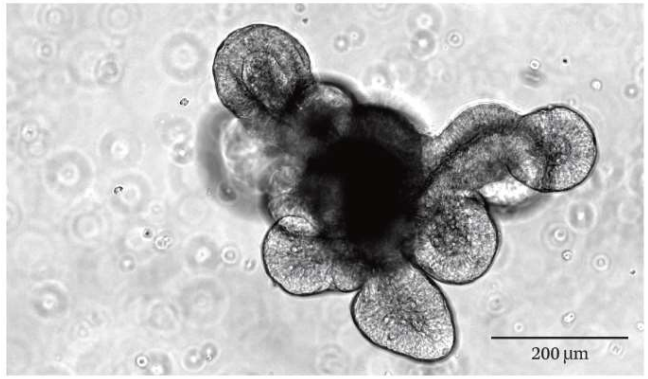


Figure 1. Light microscope visualization of a mouse intestinal organoid.
Source: STEMCELL Technologies

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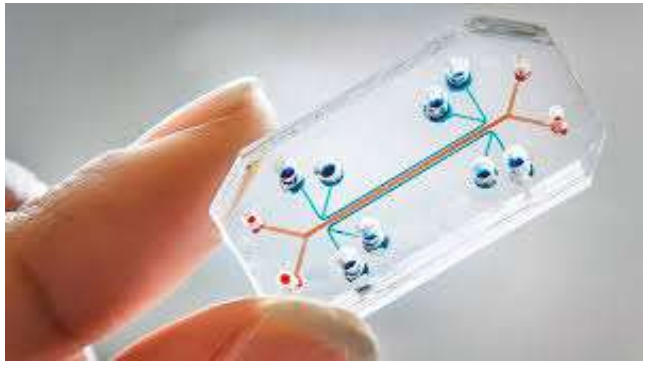
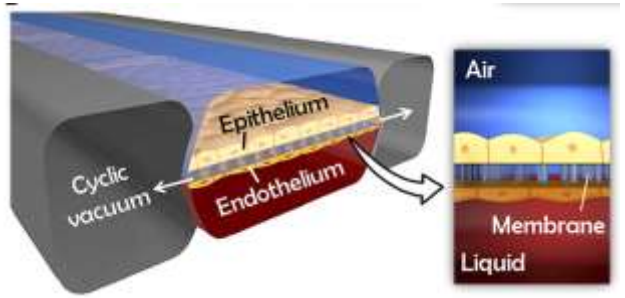
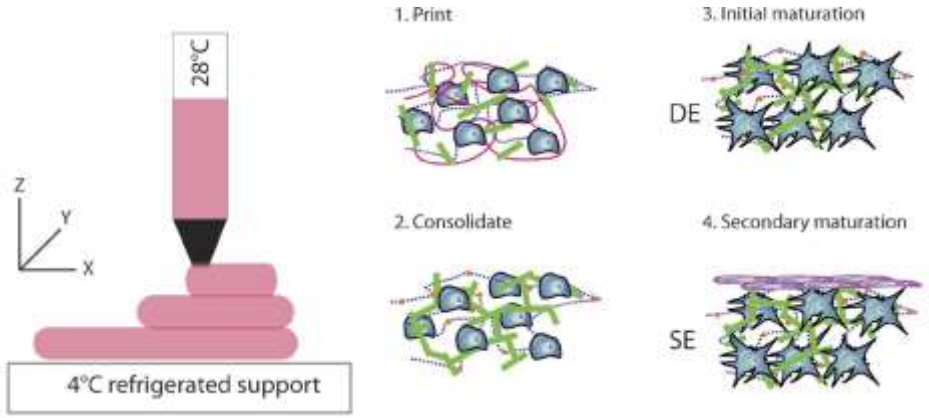
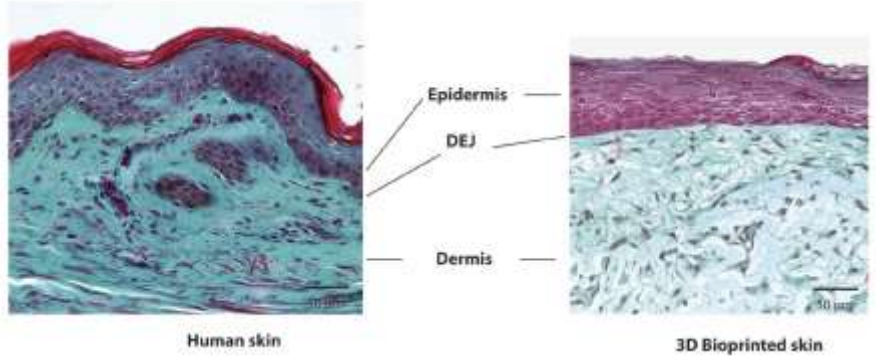
Organs on chip





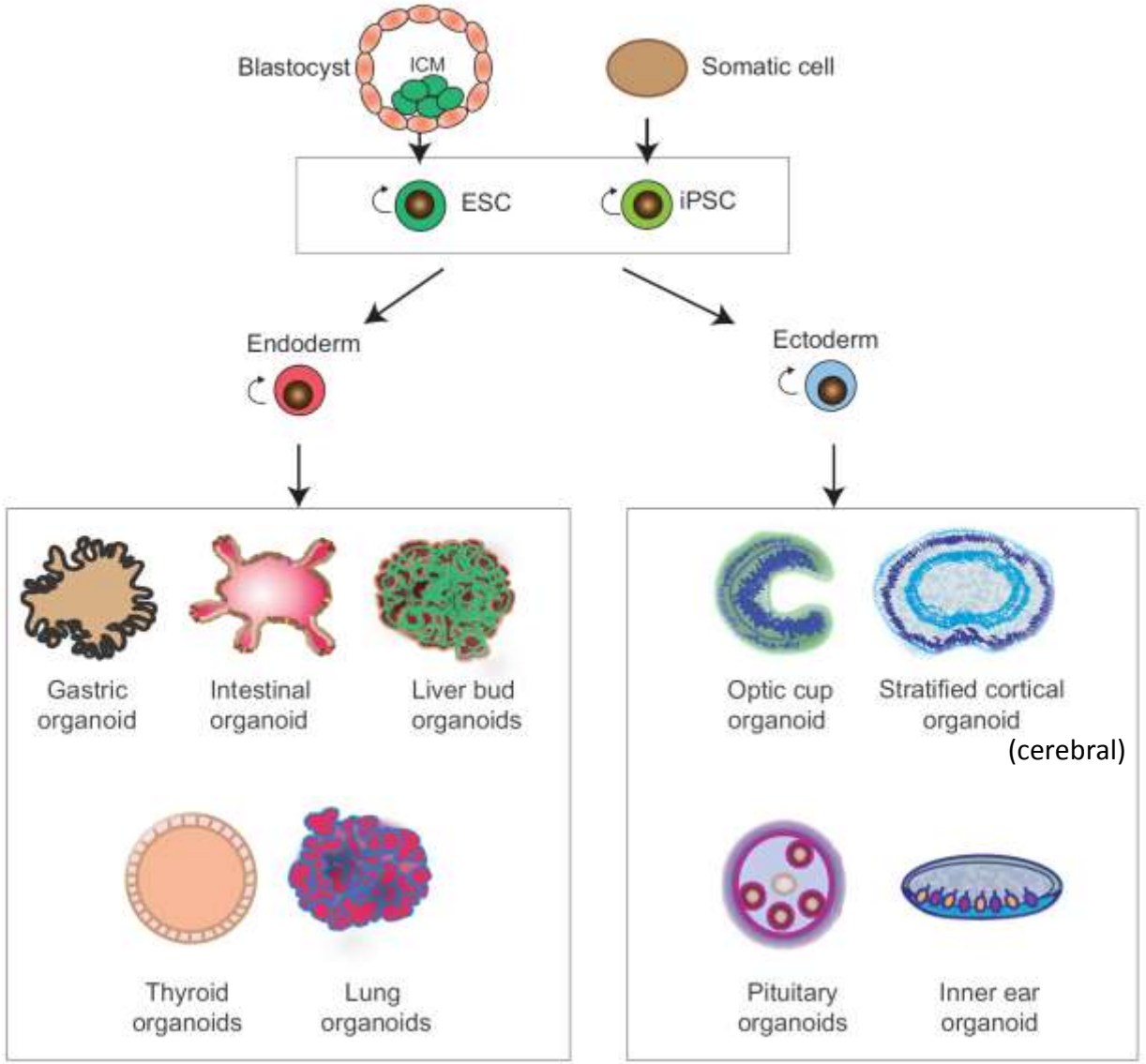
3d-bioprinted tissues





These two strategies are not “self-organized systems”, and are commonly generated from differentiated cells.

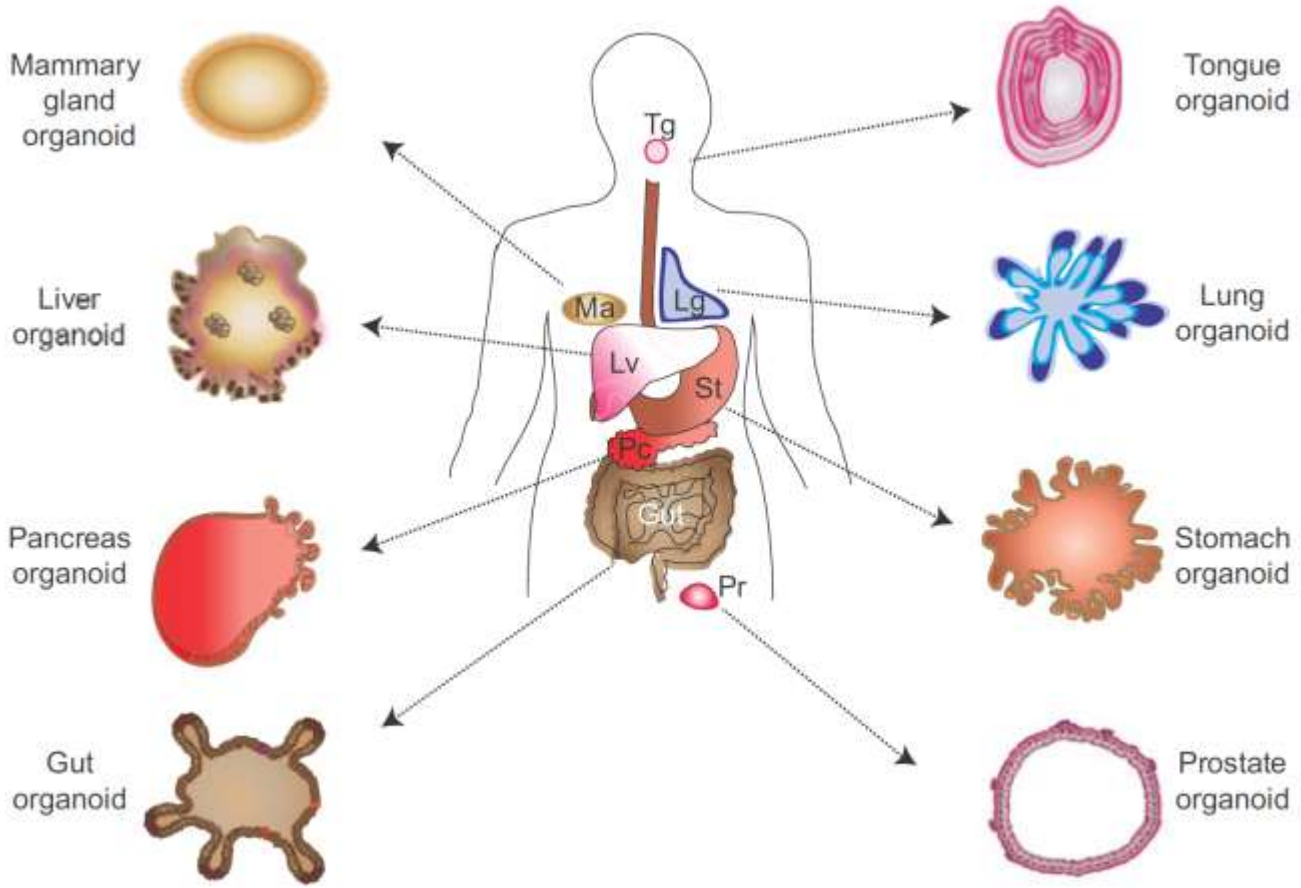
Organoids are generated from pluripotent stem cells…



Pluripotent stem cell (PSC)-derived organoids. PSCs [embryonic stem cells (ESCs) or induced PSCs (iPSCs)] can be derived into the different germ layers (endoderm, mesoderm and ectoderm) in vitro with specific stepwise differentiation protocols. After the initial germ layer specification, cells are transferred into 3D systems and generate organoids that faithfully recapitulate ex vivo the developmental steps that occur in vivo.

Meritxell Huch and Bon-Kyoung Koo; Development (2015)

…or from Adult stem cells retrieved in endodermal-derived organs



Adult primary tissues derived from endodermal organs harboring cells with stem cell potential have been cultured in vitro into adult stem cell (AdSC)-derived organoids.

Organoids can be derived from both isolated adult stem/progenitor cells or from isolated fragments of tissue from the corresponding organ (e.g. intestinal crypts, liver or pancreas ducts).

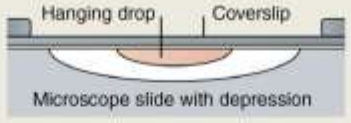
In these conditions, the cells expand long-term in culture, while maintaining their genetic stability and commitment to their tissue of origin. Transdifferentiation has not yet been observed. These cultures can be used to study stem cell biology, as models of adult functional tissue and to study somatic mutational processes. Lg, Lung; Lv, Liver; Ma, mammary gland; Pc, Pancreas; Pr, Prostate; St, Stomach; Tg, Tongue.

Meritxell Huch and Bon-Kyoung Koo; Development (2015)

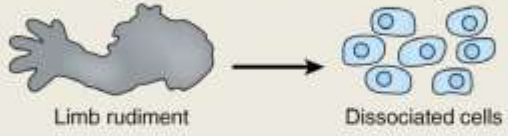
Timeline of techniques and experiments leading to the current organoid field

Marina Simian and Mina J. Bissell; JCB 2017

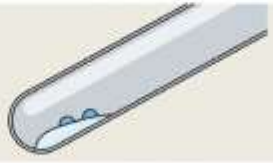
1906 Harrison
Hanging drop cell culture method



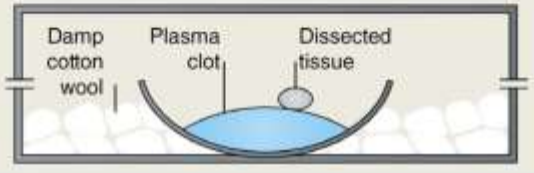
1914 Thomson
Definition of cultures as "controlled" vs "uncontrolled" when tissue organization and functionality are lost



1926 Strangeways & Fell
Tube cultures

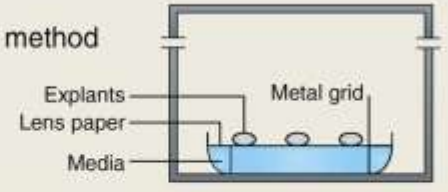


1929 Fell & Robison
Watch glass method

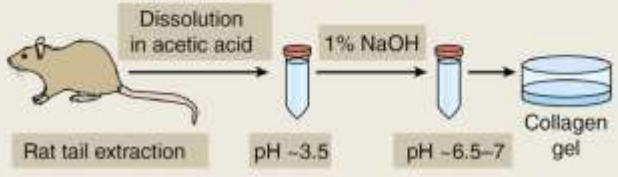


1952 Moscona & Moscona
Dissociation and aggregation of cells from organ rudiments of the early chick embryo

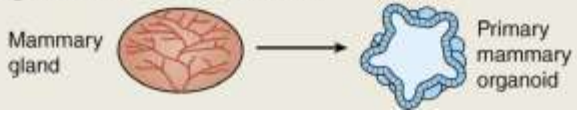
1954 Trowell
Grid cell culture method



1956 Ehrmann & Gey
Reconstituted collagen from rat tails



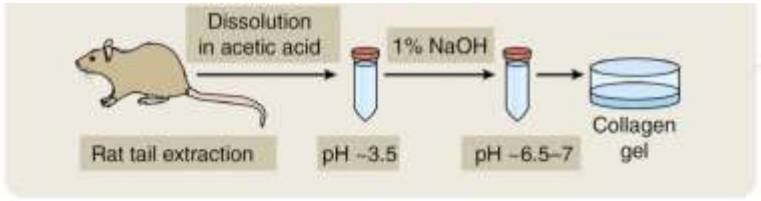
1957 Lasfargues
Dissociation of mammary glands into organoids using collagenase from *Clostridium*



1906
1914
1926
1929
1952
1954
1956
1957

Timeline of techniques and experiments leading to the current organoid field

Marina Simian and Mina J. Bissell; JCB 2017



1956

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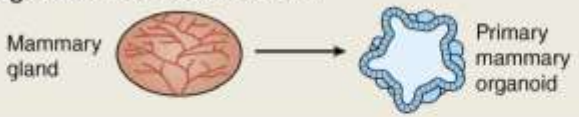
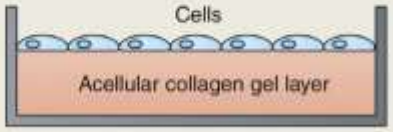


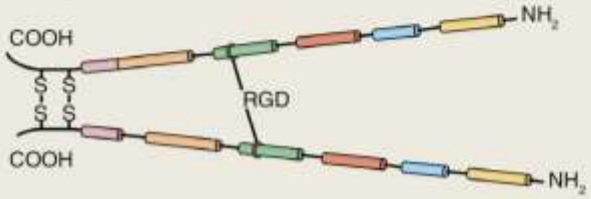
1957

1960-1977
Culture of different cell types on collagen I gels



1960

1973 Gahmberg & Hakomori; Ruoslahti et al.; Hynes
Discovery of Fibronectin



1973

1975 Michalopoulos and Pitot
Differentiation of hepatocytes on floating collagen gels

1977 Emerman and Pitelka
Culture of differentiated mammary epithelial cells on floating collagen gels

1975

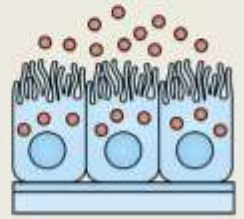
1977 Orkin
Gel isolated from matrix of chondrosarcomas, EHS, known today as Matrigel

1977

1979 Timpl et al.
Characterization of laminin

1979

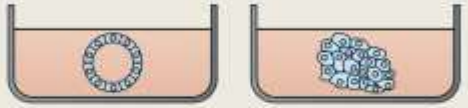
1984, 1985 Lee et al.
Basal and luminal polarity is established when cells are on floating collagen gels,as shown by milk protein synthesis and secretion into the medium.



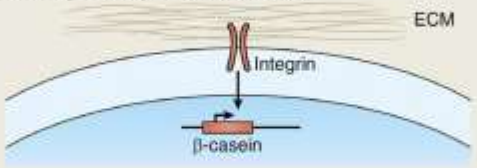
1984

1987 D.M. Bissell et al.; Li et al.
Demonstration of the functional use of laminin-rich gels to support hepatocellular function or mammary gene expression.

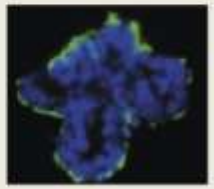
1989 Barcellos-Hoff et al. **1992** Petersen et al.
Use of a laminin-rich matrix to develop assays of mammary morphogenesis and to distinguish between healthy and malignant human epithelial cells.



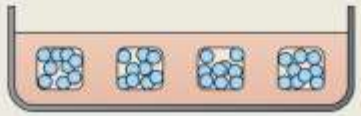
1991 Streuli et al.
Integrins regulate gene expression



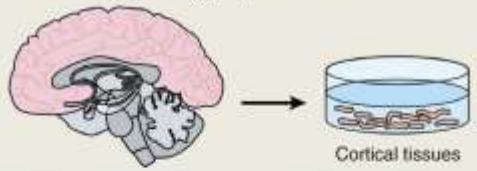
2001 Simian et al.
Use of 3D collagen cultures to study the mechanisms of mammary gland branching morphogenesis



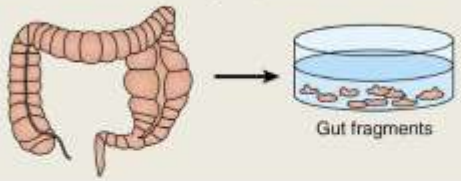
2006 Nelson et al.
Micropattern gels provide positional cues that establish the range of action of TGF-β in morphogenesis vs invasion



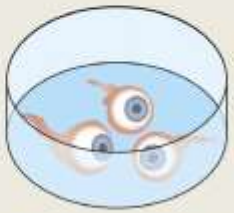
2008 Eiraku et al.
Self-organized formation of polarized cortical tissues from ESCs using 3D aggregation cultures



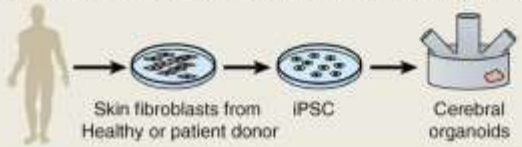
2009 Sato et al.
"Mini-guts": a culture system allows growth of epithelial organoids from a single Lgr5-positive stem cell



2012 Nakano et al.
Formation of a self-organized optic cup structure from human ESCs in 3D culture



2013 Lancaster et al.
Human brain organoids are generated from iPSCs derived from cells from a patient with microcephaly.



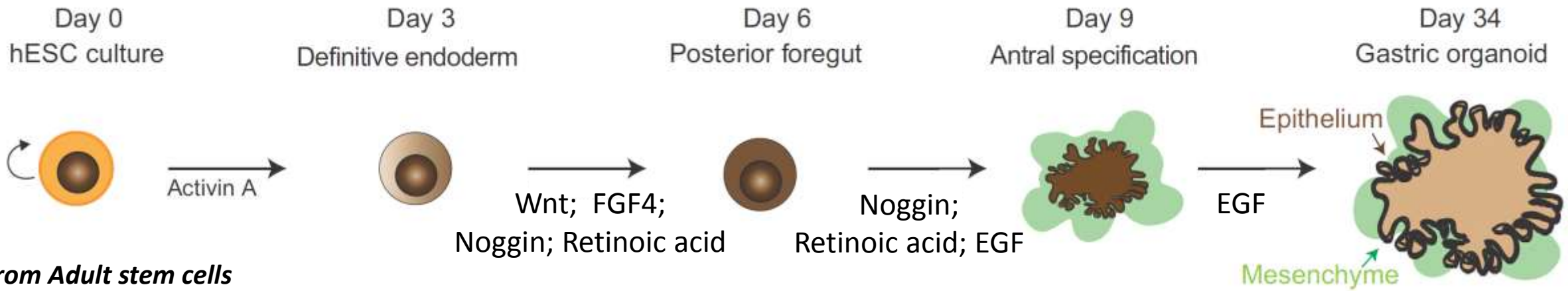
1987
1989
1991
2001
2006
2008
2009
2012
2013

Marina Simian and Mina J. Bissell; JCB 2017

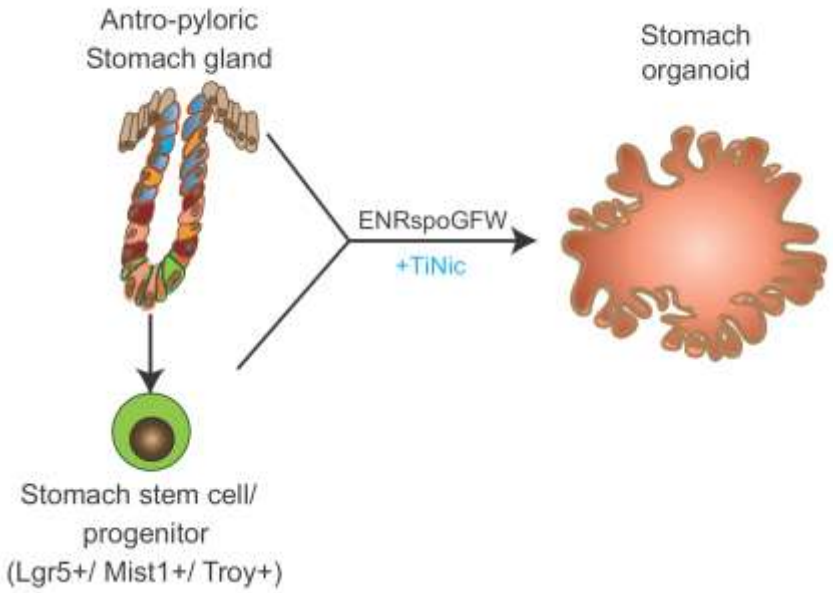
Example of currently existing organoids:

Stomach organoids

A ***From pluripotent stem cells***



B ***From Adult stem cells***



Gastric organoids derived from PSCs (A) or AdSCs (B). When AdSCs are used as source, the gland is formed by ***Lgr5+ stomach stem cells***, chief (zymogen-producing cells) cells and a transit amplifying compartment, while the pit region faces the lumen of the organoid structure. Adapted from Barker et al. (2010a). Ti, Tgfb inhibitor; Nic, Nicotinamide. Ti and Nic are only needed for human stomach AdSC-cultures, but not for mouse. E, EGF; N, noggin; Rspo, Rspondin; G, gastrin; F, FGF10, W, Wnt.

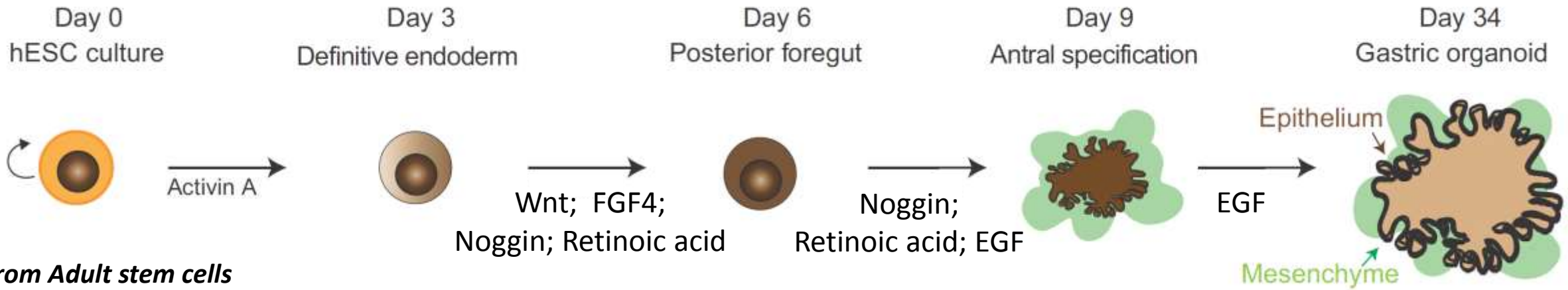
Meritxell Huch and Bon-Kyoung Koo; Development (2015)

Example of currently existing organoids:

Stomach organoids

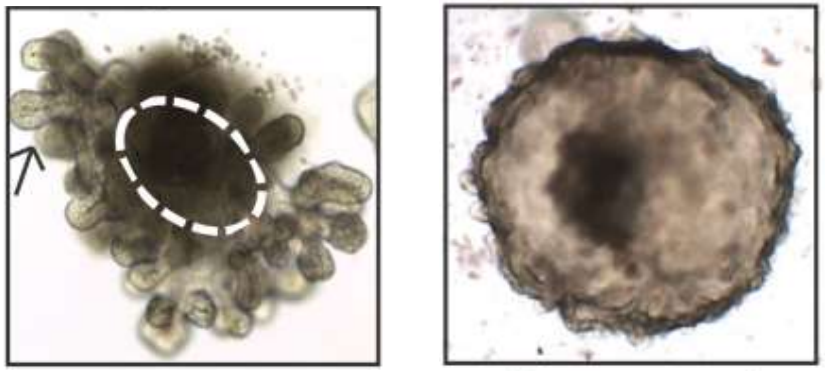
A

From pluripotent stem cells



From Adult stem cells

The gastric organoids self-organize into 3D structures that contain a ***glandular-like domain (arrow)*** and a ***central empty lumen*** (pit region, ***dashed white circle***). The empty lumen gets filled with the dead cells as they are pushed from the gland base to the pit region and reach the top of the lumen, in a similar manner as they do in vivo, during normal tissue homeostasis. Enteroendocrine cells are scattered all over the organoid structure.



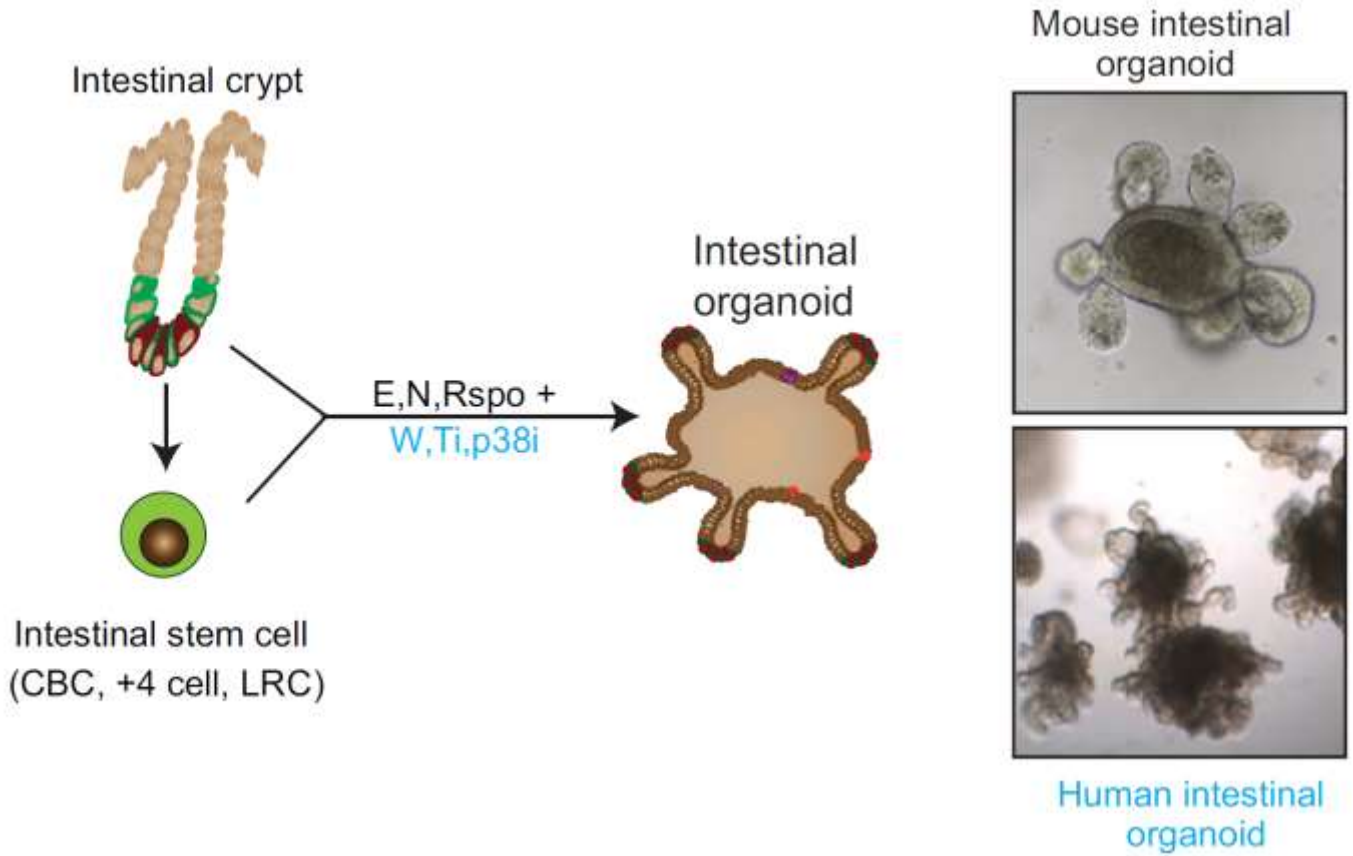
Mouse stomach organoid

Human stomach organoid

Meritxell Huch and Bon-Kyoung Koo; Development (2015)

Example of currently existing organoids:

Intestinal organoids

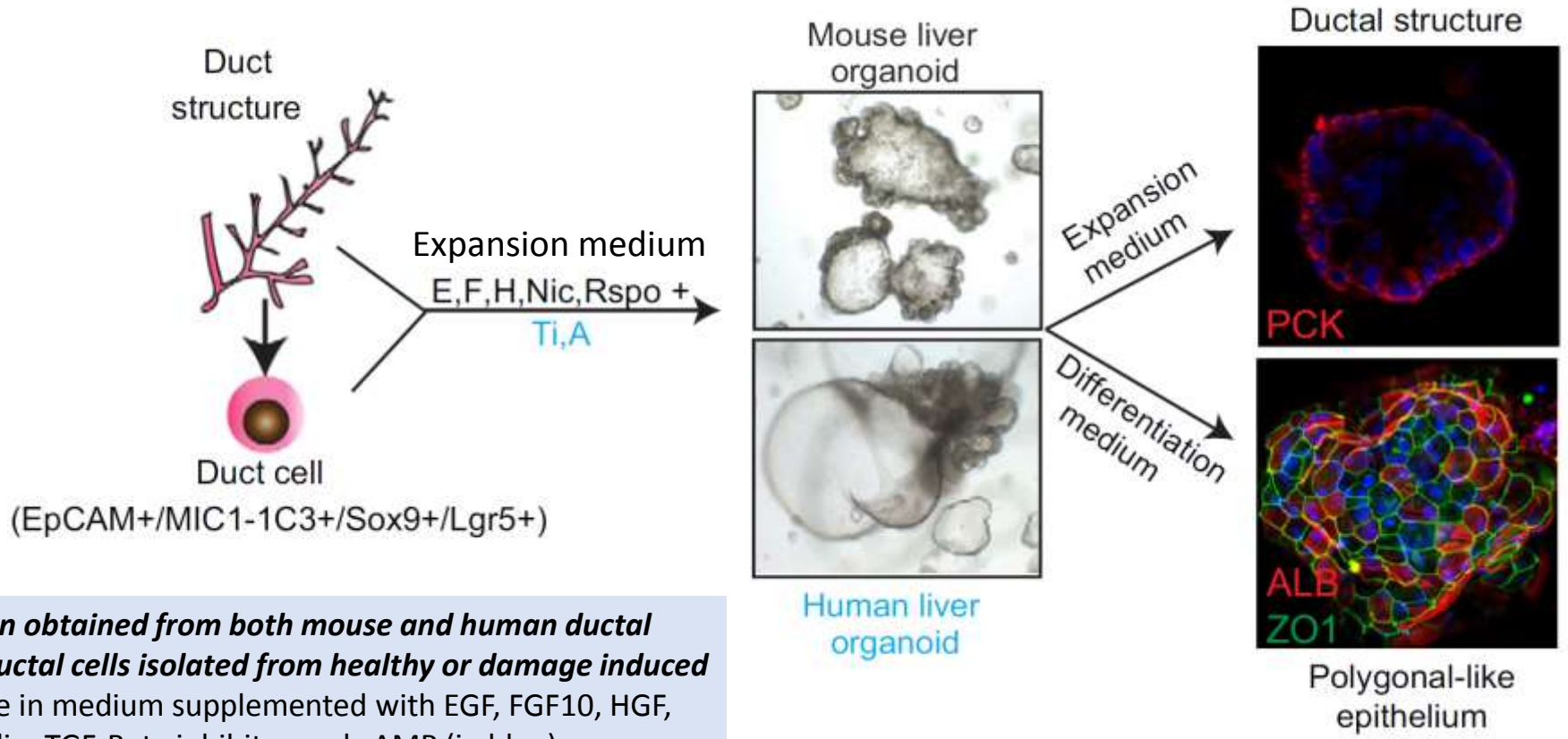


Small-intestine organoids are obtained from either adult intestinal crypts or adult intestinal stem cells (isolated from the bottom of the crypts by FACS using specific markers). Sorted cells or crypts are cultured in medium with EGF, Noggin and Rspondin. Intestinal stem cells [crypt base columnar cells, label-retaining cells (LRC), +4 cells as well as Dll1+ cells] have all been successfully grown into organoids in the presence of EGF, Noggin, Rspondin and addition of Wnt for the first days. Addition of TGF-beta inhibitor, p38 inhibitor and Wnt (in blue) is required only for the growth of human gut organoids.

Meritxell Huch and Bon-Kyoung Koo; Development (2015)

Example of currently existing organoids:

Liver organoids

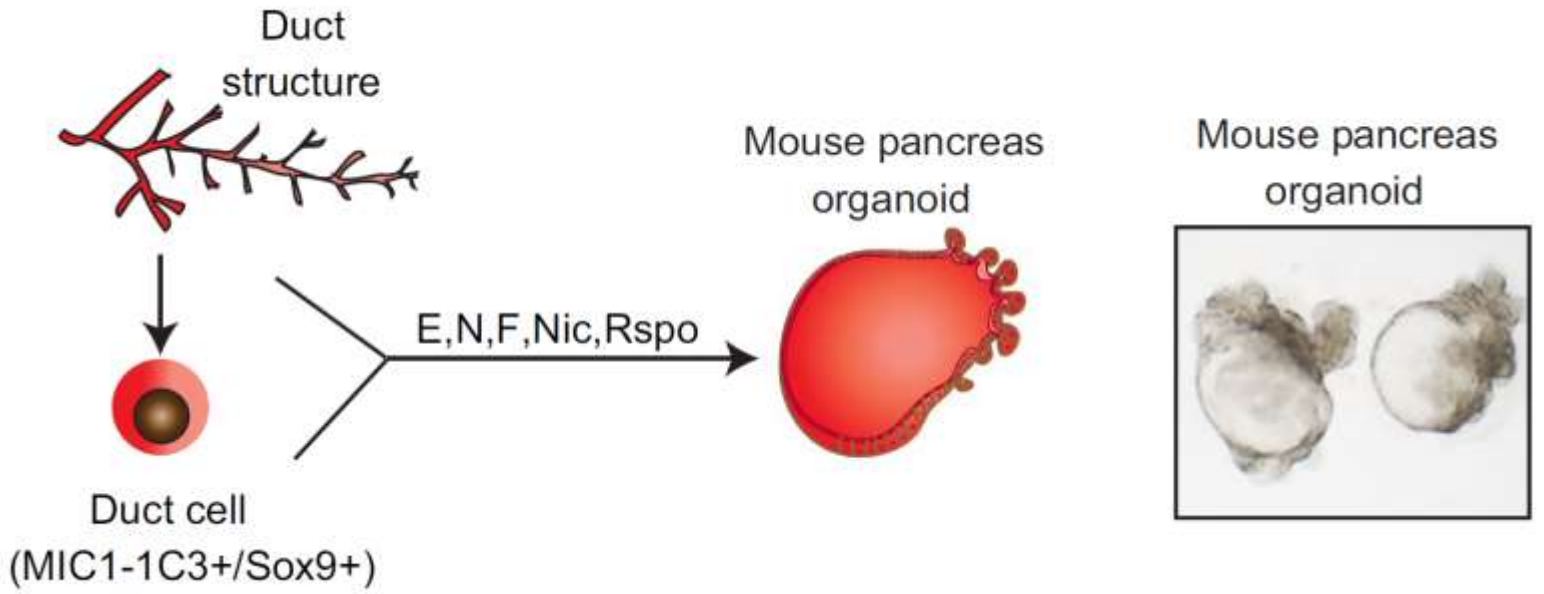


Liver organoids have been obtained from both mouse and human ductal structures or from liver ductal cells isolated from healthy or damage induced livers after culturing these in medium supplemented with EGF, FGF10, HGF, Nicotinamide and Rspondin. TGF-Beta inhibitor and cAMP (in blue) are required for the growth of human liver organoids. Under such expansion medium conditions the cells expand in culture as ductal structures (PCK, pan-cytokeratin, red). When transferred to differentiation medium (bottom panel on right hand), the ductal cuboidal epithelium differentiates into a polygonal-like epithelium that expresses hepatocyte markers (albumin, red; ZO1, green).

Meritxell Huch and Bon-Kyoung Koo; Development (2015)

Example of currently existing organoids:

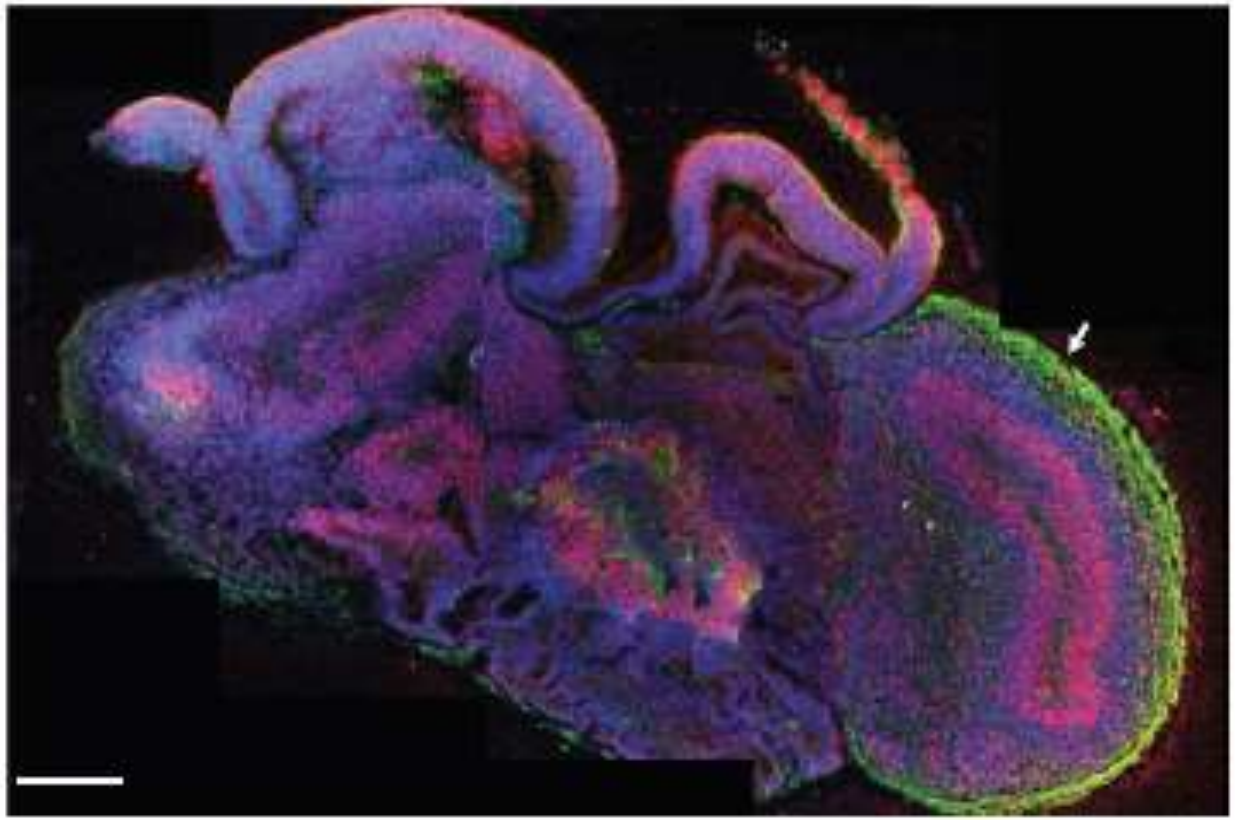
Pancreas organoids



Mouse pancreas organoids derived from either duct structures or isolated ductal cells grow into pancreas organoids that expand as ductal cells in culture. E, EGF; F, FGF10; Nic, Nicotinamide; N, noggin; Rspo, Rspondin.

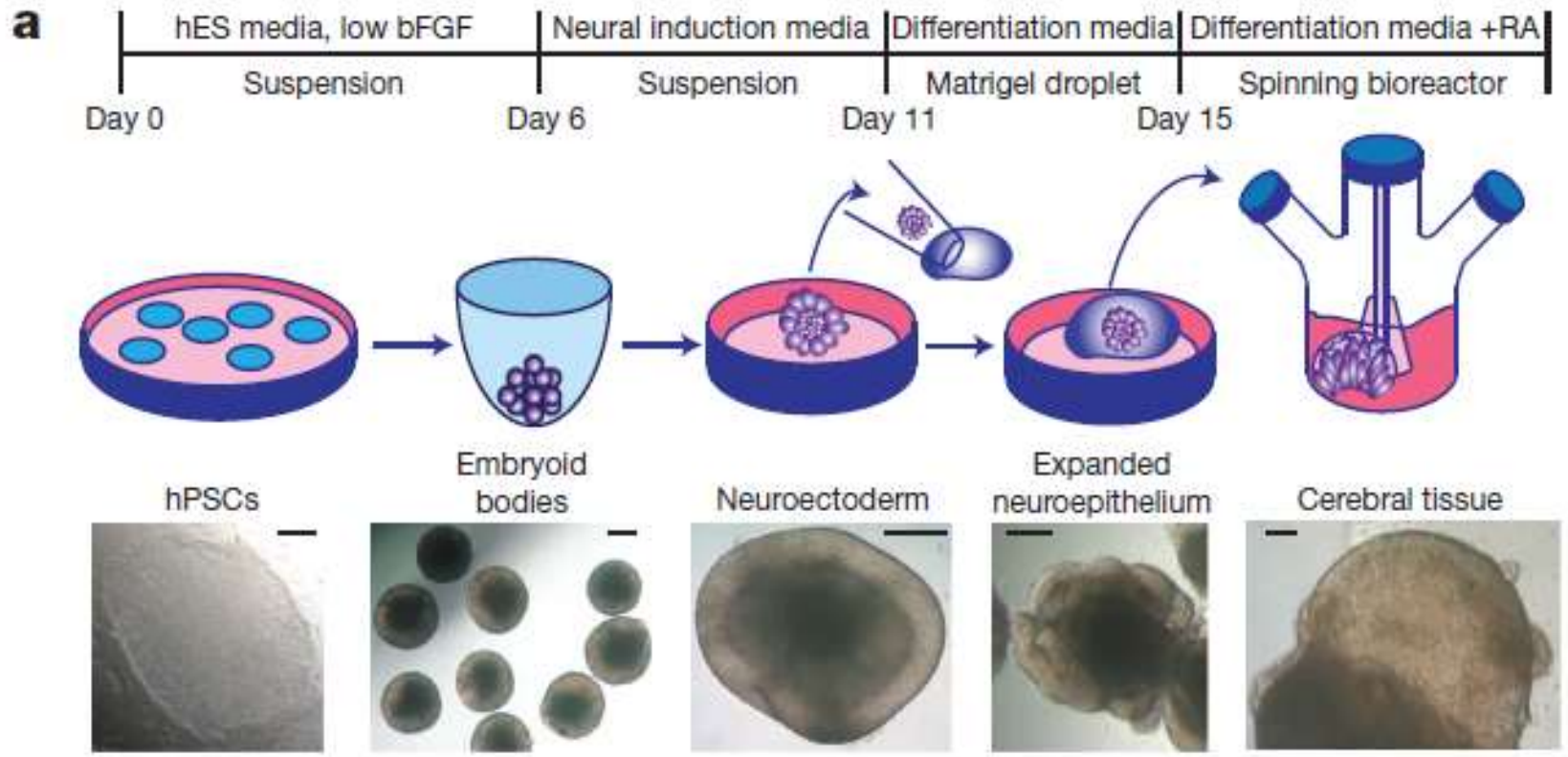
Meritxell Huch and Bon-Kyoung Koo; Development (2015)

Modeling nervous tissue complexity with cerebral organoid cultures



Lancaster et al; Nature (2013)

Cerebral organoids



a, Schematic of the culture system requiring the embedding of embryoid bodies on Matrigel droplets and continuous agitation culturing with the help of spinning bioreactors.

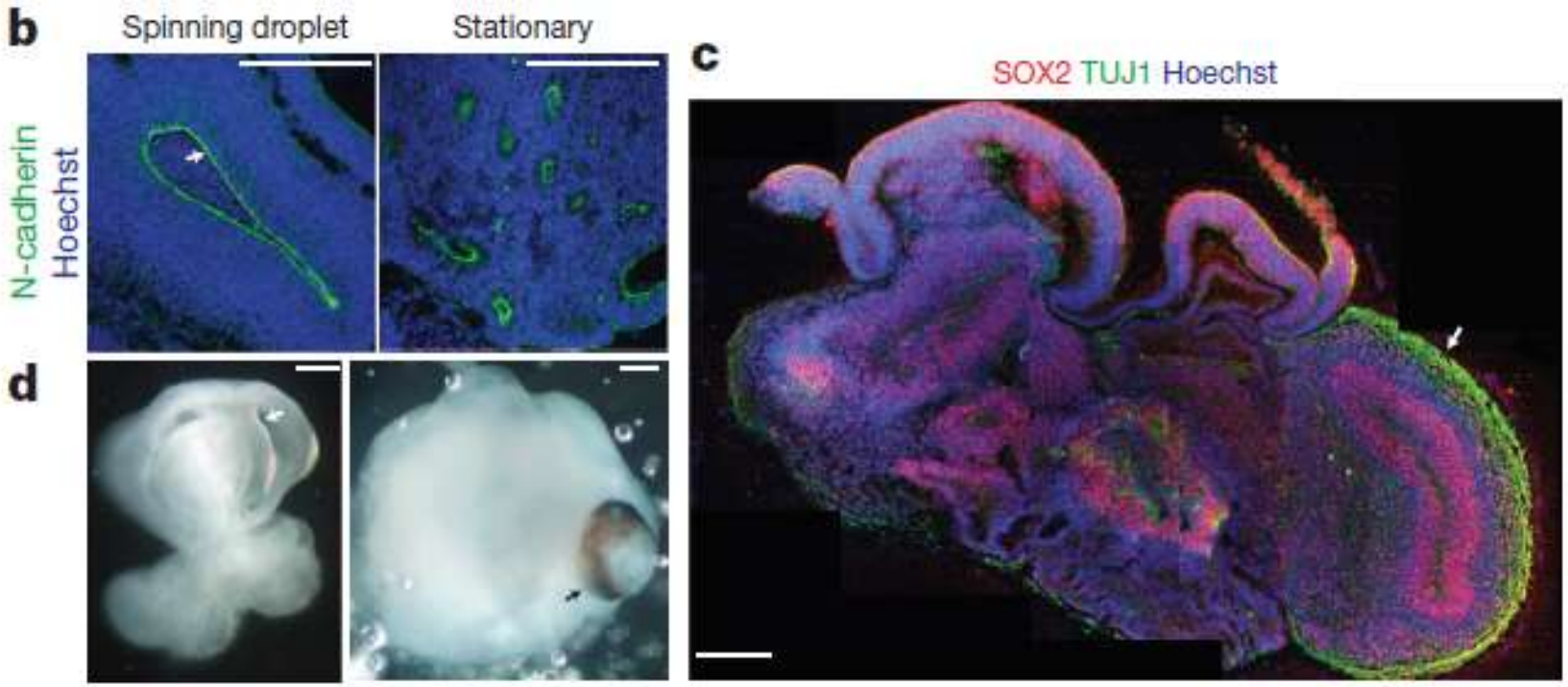
ARTICLE *September 2013*

doi:10.1038/nature12517

Cerebral organoids model human brain development and microcephaly

Madeline A. Lancaster[1], Magdalena Renner[1], Carol-Anne Martin[2], Daniel Wenzel[1], Louise S. Bicknell[2], Matthew E. Hurles[3], Tessa Homfray[4], Josef M. Penninger[5], Andrew P. Jackson[2] & Juergen A. Knoblich[1]

Cerebral organoids



b, Neuroepithelial tissues generated using this approach (left panel) exhibited large fluid-filled cavities and typical apical localization of the neural N-cadherin (arrow). These tissues were larger and more continuous than tissues grown in stationary suspension without Matrigel (right panel). **c,** Sectioning and immunohistochemistry revealed complex morphology with heterogeneous regions containing neural progenitors (Sox2, red) and neurons (Tuj1, green) (arrow). **d,** Low magnification bright field images revealing fluid-filled cavities reminiscent of ventricles (white arrow) and retina tissue, as indicated by retinal pigmented epithelium (black arrow). Scale bars: 200 µm.

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Cerebral organoids

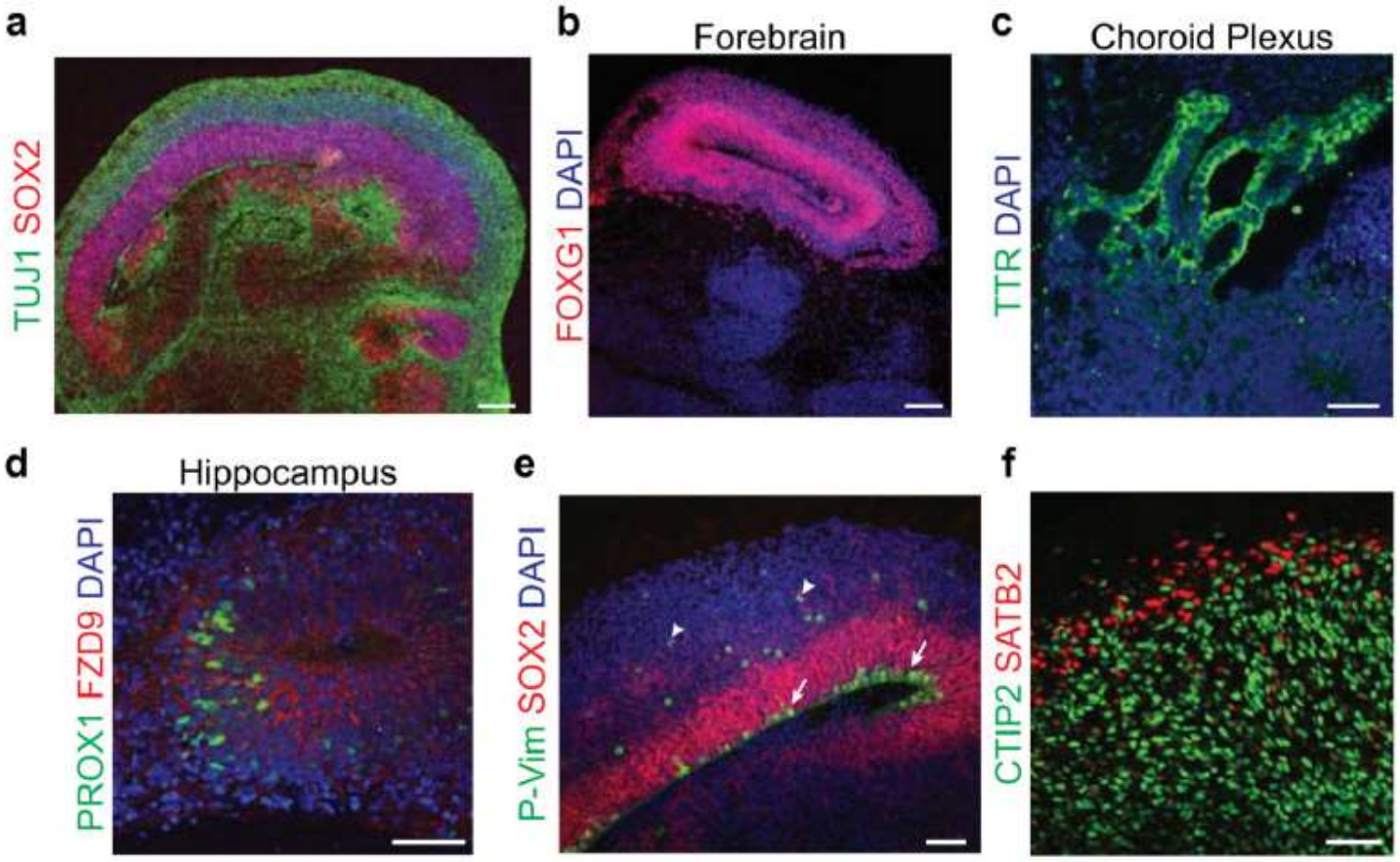

Figure 4. Staining for brain regions and neuronal cell identities
a. Staining for neurons (TUJ1, green) and progenitors (SOX2, red) in a large continuous cortical tissue within an organoid. Note the organized apical progenitor zone surrounded by basally located neurons. **b.** A forebrain region of an organoid staining positive for the marker FOXG1 (red). **c.** Choroid plexus stains positive for the marker TTR (green) and displays convoluted cuboidal epithelium. **d.** Hippocampal regions stain positive for the markers PROX1 (green) and FZD9 (red), although the cells fail to spatially organize into recognizable dentate gyrus and CA regions. **e.** Staining for mitotic radial glia (P-Vimentin, P-Vim, green) in a cortical region reveals inner radial glia undergoing mitosis at the apical membrane (arrows), while outer radial glia undergo mitosis outside the ventricular zone (arrowheads). All radial glia are marked by SOX2 (red). **f.** Staining for cortical layer identities of advanced organoids (75 days). Later-born superficial layer identity (SATB2, red) neurons populate more superficial regions of the organoid, while early-born deep layer identity (CTIP2, green) neurons populate deeper regions of the organoid. DAPI in a-e labels nuclei (blue). Samples in a-e are 30-35 days after initiation of the protocol. Scale bar is 100 μm in a-b and 50 μm in c-f.

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Bio-reactor solutions for culturing brain organoids

ARTICLE *Nature Sept. 2013*

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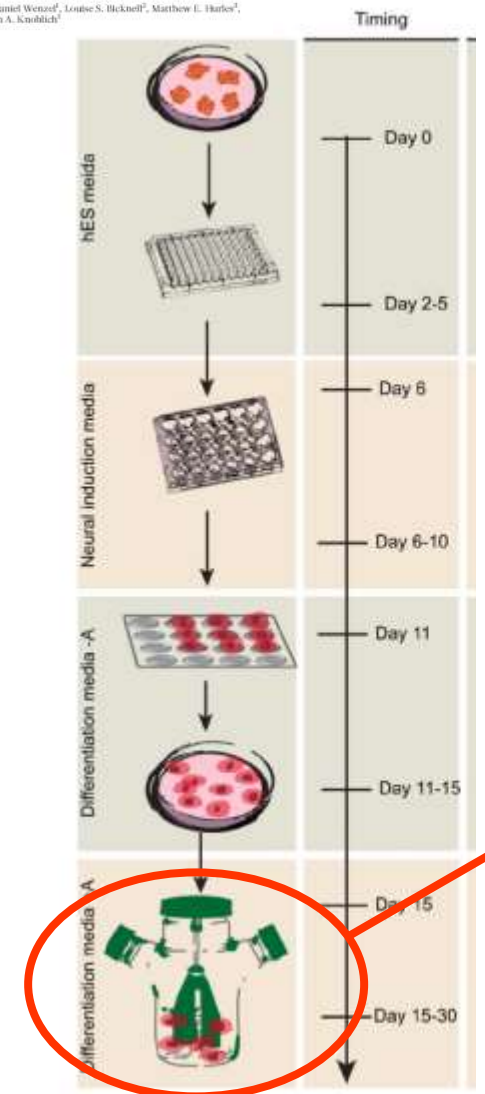

As described in the original article by M. Lancaster et al; (Nature 2013), bioreactors in use require 125 ml medium, which is changed twice a week. Hence for assays covering ~ 3months, it becomes rather expensive. In addition, the size of such bioreactors occupies large space on incubators.

Bio-reactor solutions for culturing brain organoids

ARTICLE *Nature Sept. 2013*

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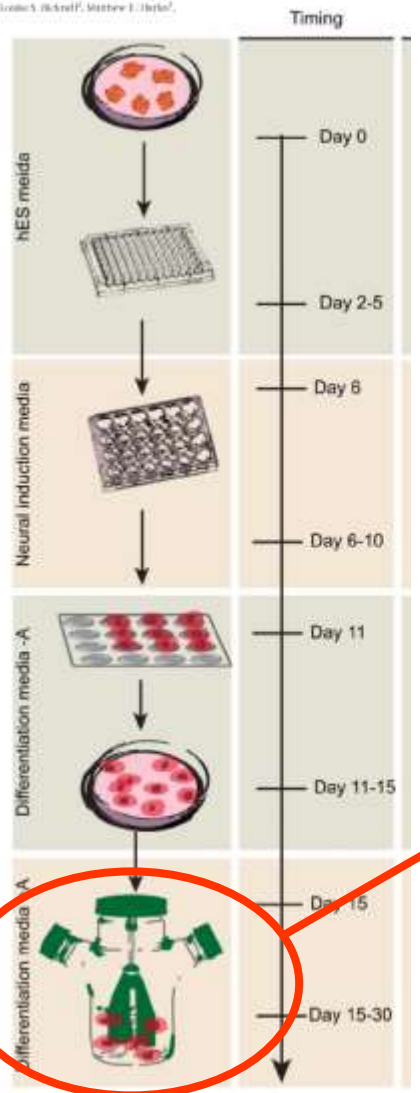
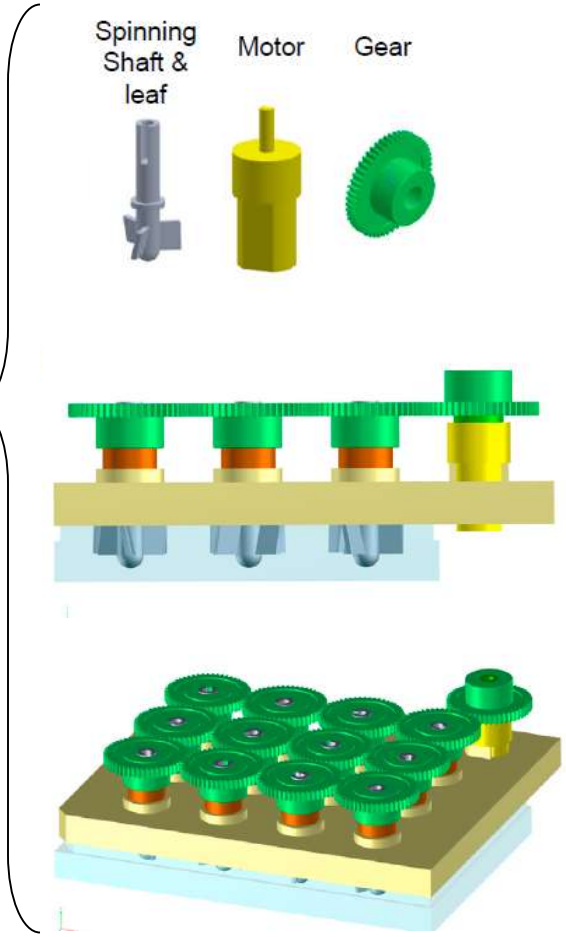


May 2016

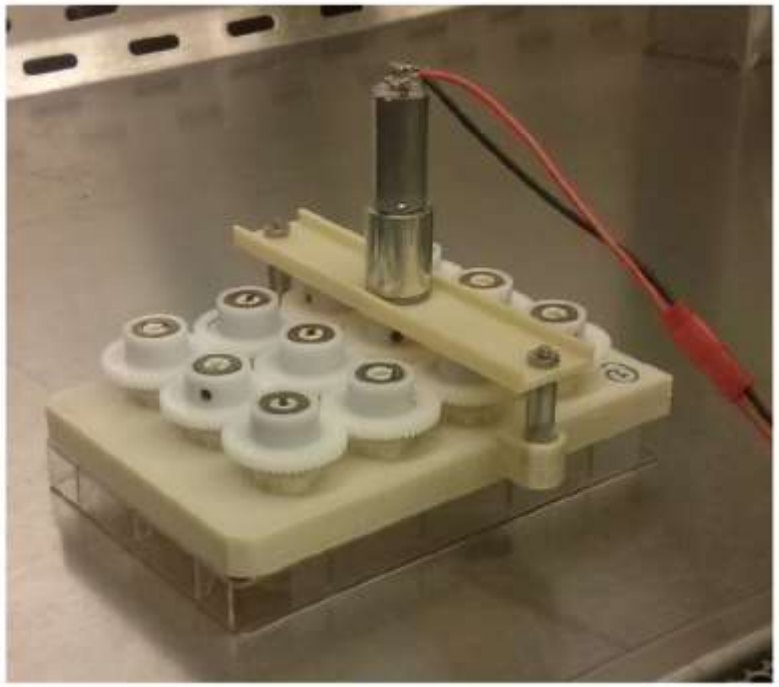
Cell

Brain-Region-Specific Organoids Using Mini-bioreactors for Modeling ZIKV Exposure



12-well plate

Bio-reactor solutions for culturing brain organoids



SpinU Bioreactor-based Forebrain Organoid Culture System.
(A) Computer-aided design drawings of 12-well version SpinU bioreactor and individual parts.

Nat Protocols 2018

PROTOCOL

Generation of human brain region–specific organoids using a miniaturized spinning bioreactor

Xuyu Qian[1,2,7], Fadi Jacob[1,3,7], Mingxi Max Song[4], Ha Nam Nguyen[5], Hongjun Song[1,6] & Guo-li Ming[1,6]

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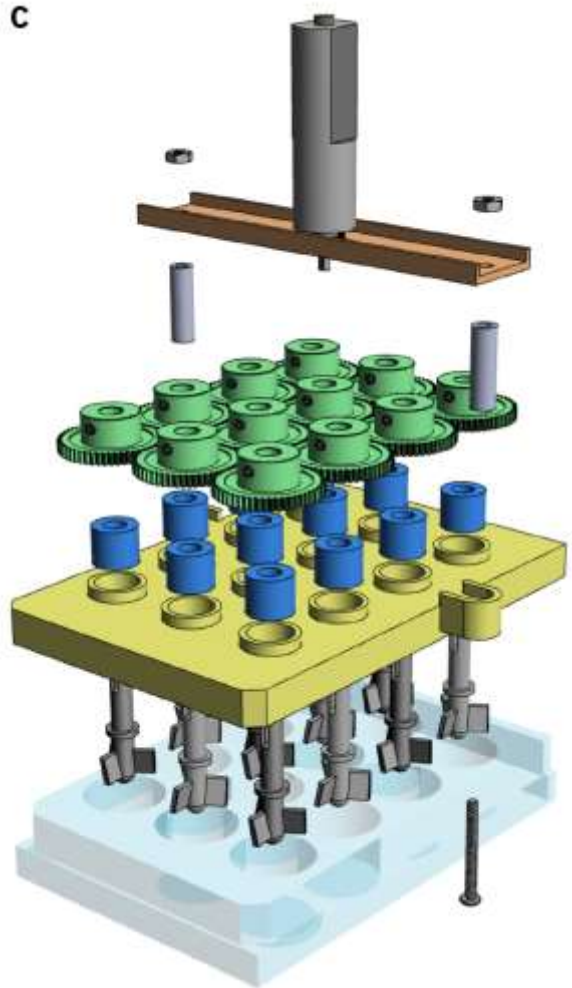
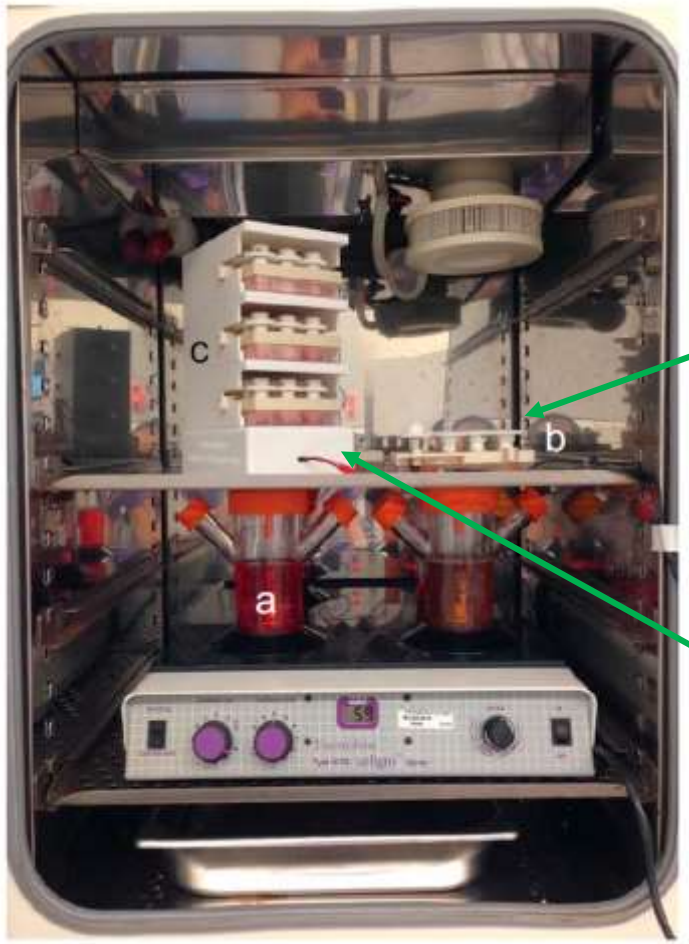
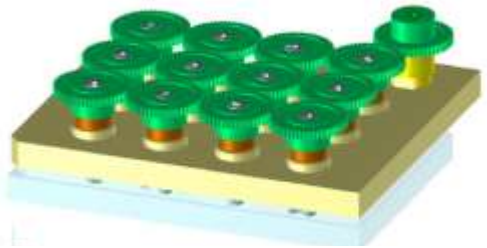
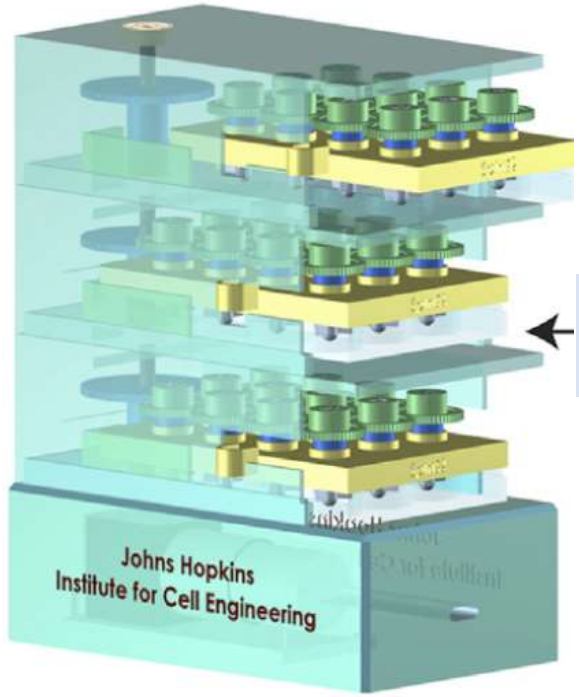


Bio-reactor solutions for culturing brain organoids







A "tower" version of the spin-U bioreactor hosting up to 3 x 12 well plates

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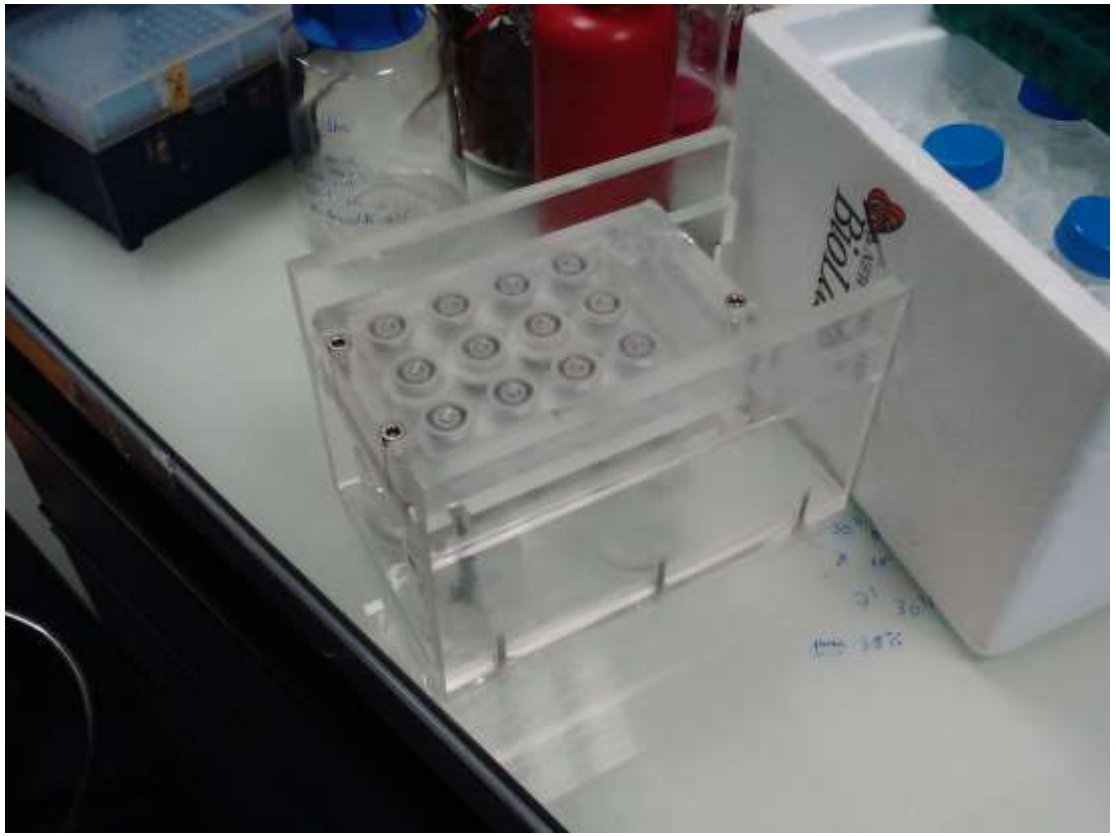
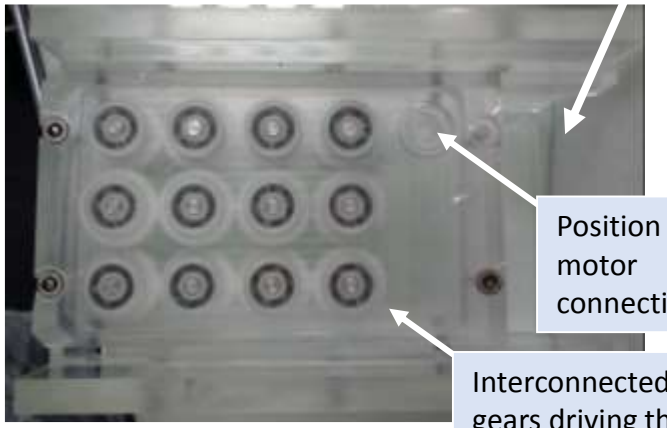
Bio-reactor solutions for culturing brain organoids

Mendoza's team SysFate home-made bioreactor

This bioreactor is an intermediate solution in size between the 12-well plate bioreactor designed by XY. Quian et al (Cell 2016; J. Hopkins Institute for cell Engineering; Baltimore USA) and the use of 125ml size spinning bioreactor used by Madeline Lancaster (Nature 2013; IMP; Austria).

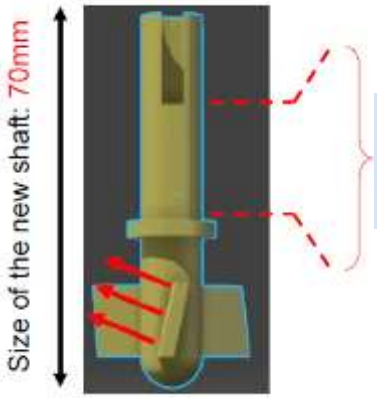


Top view



Front view

Modified spin shafts (3D printing)



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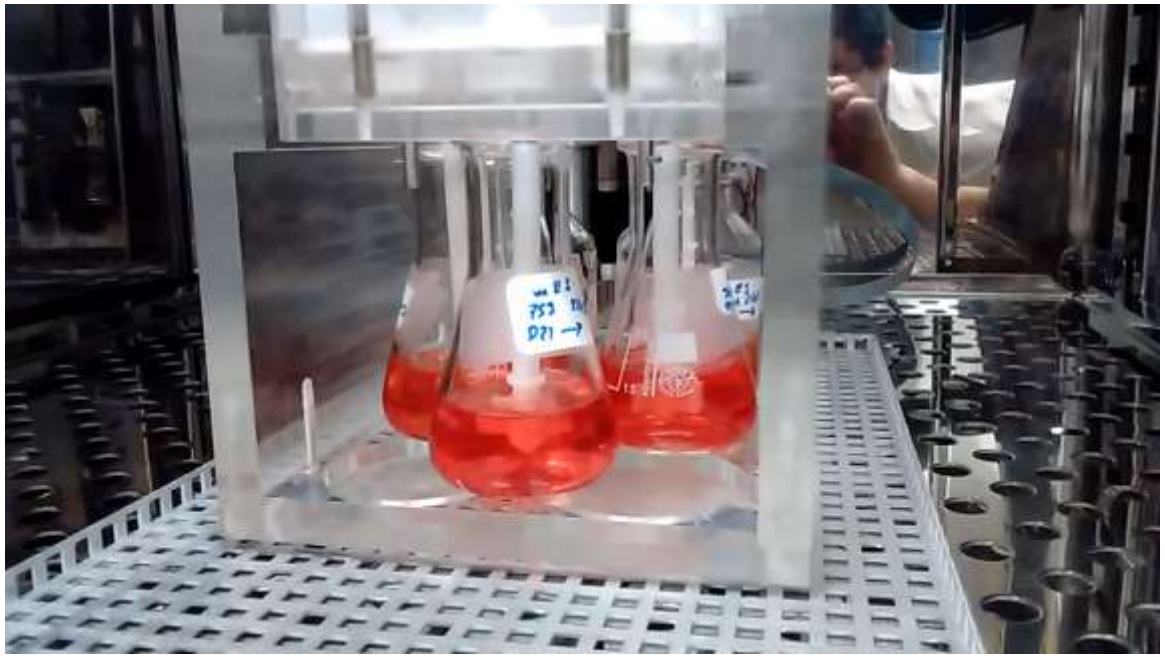

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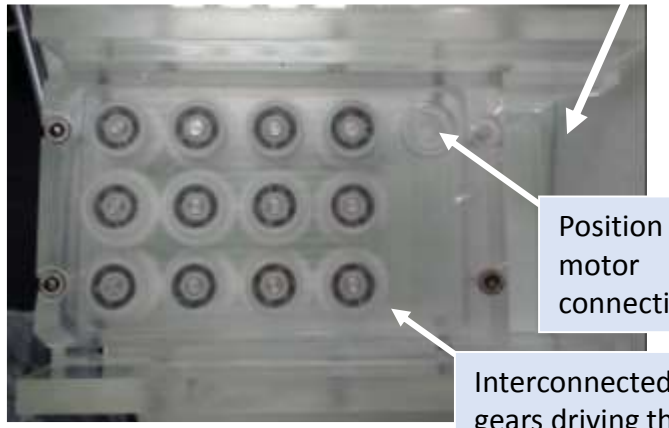
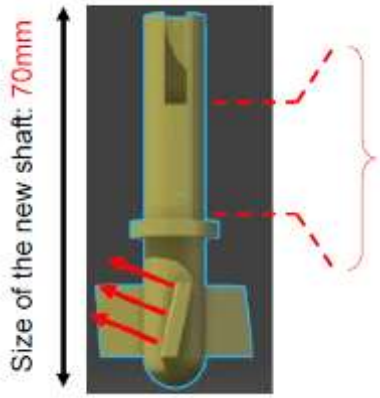
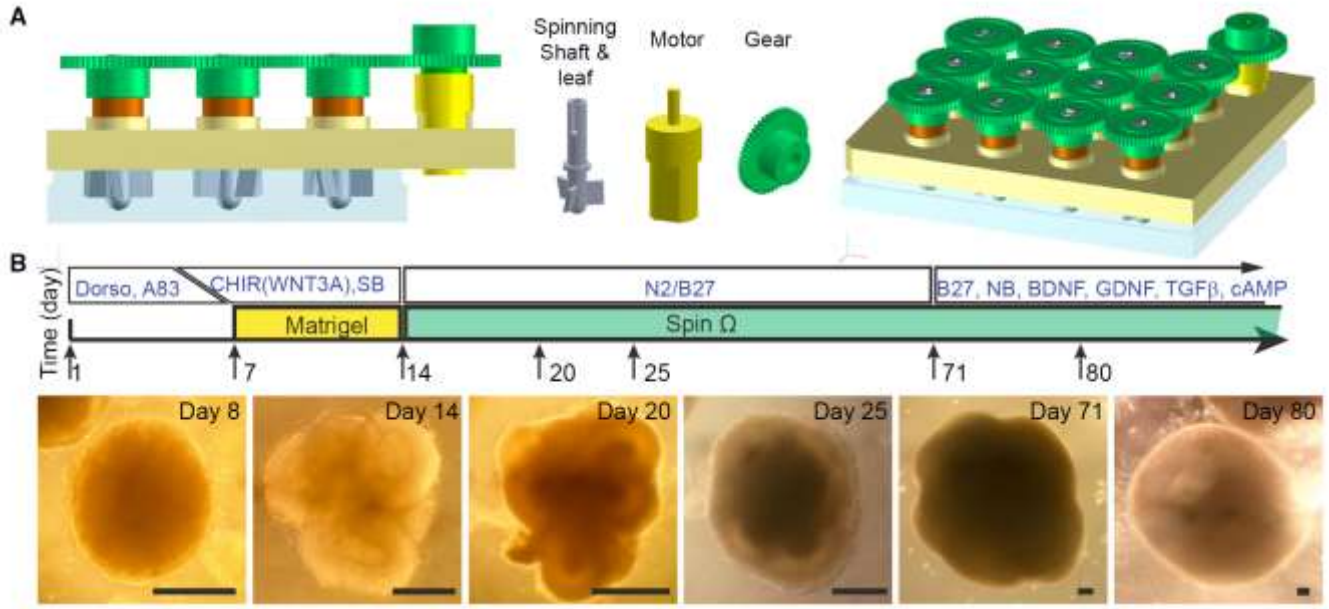


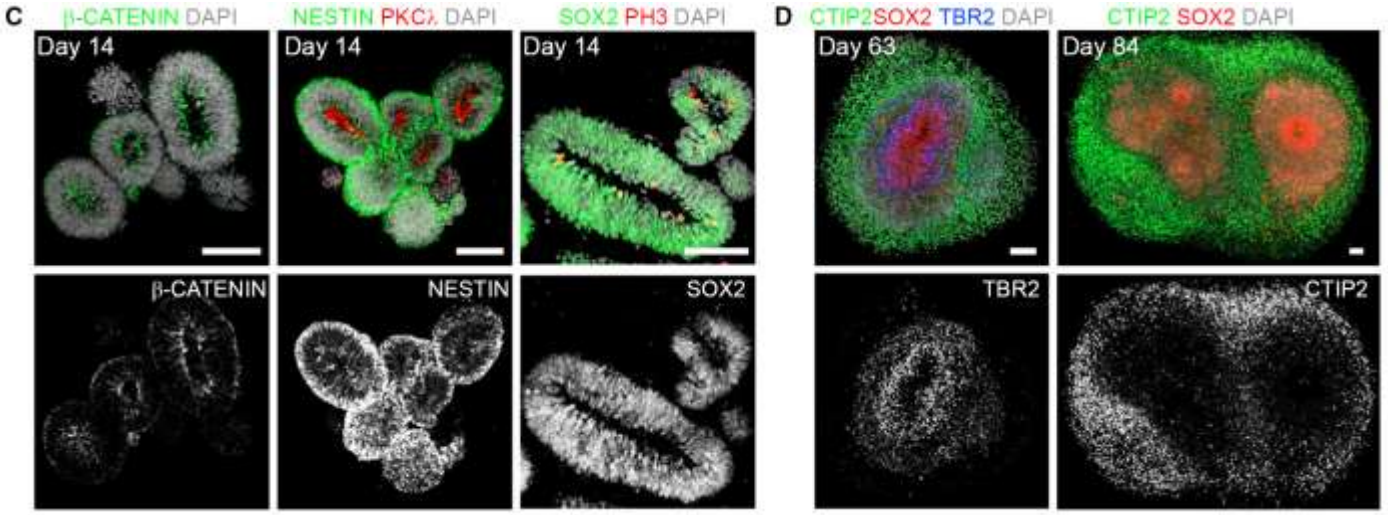
Front view

Modified spin shafts (3D printing)



Bio-reactor solutions for culturing brain organoids





SpinU Bioreactor-based Forebrain Organoid Culture System.
(A) Computer-aided design drawings of 12-well version SpinU bioreactor and individual parts.
(B) Schematic diagram of forebrain organoid protocol and sample phase images at different stages. Scale bars, 200 mm.
(C and D) Immunostaining of forebrain organoids at days 14, 63, and 84 (tiling image). Scale bars, 100 mm.

May 2016

Cell

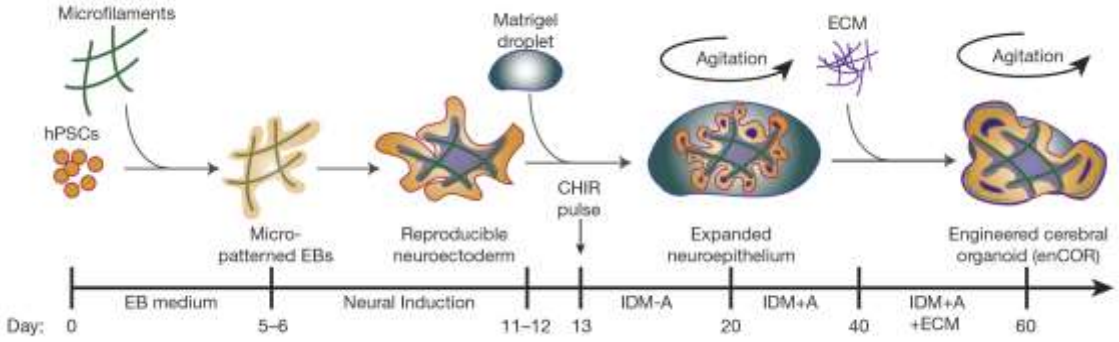
Brain-Region-Specific Organoids Using Mini-bioreactors for Modeling ZIKV Exposure

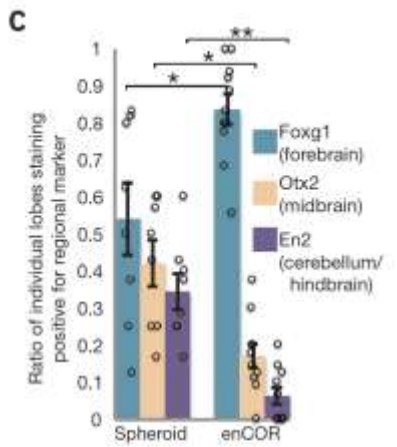
Other strategies for culturing brain organoids

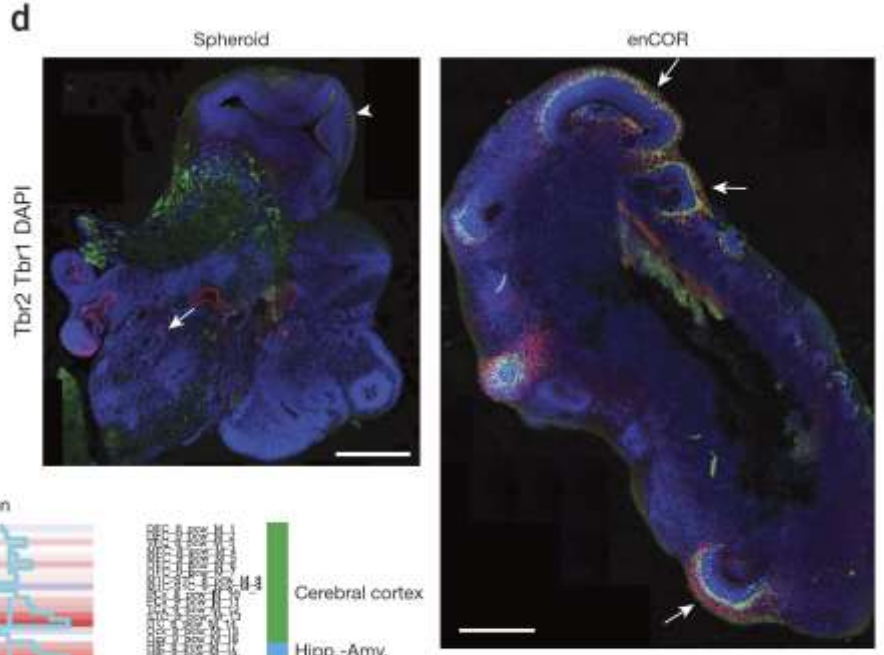
Guided self-organization and cortical plate formation in human brain organoids

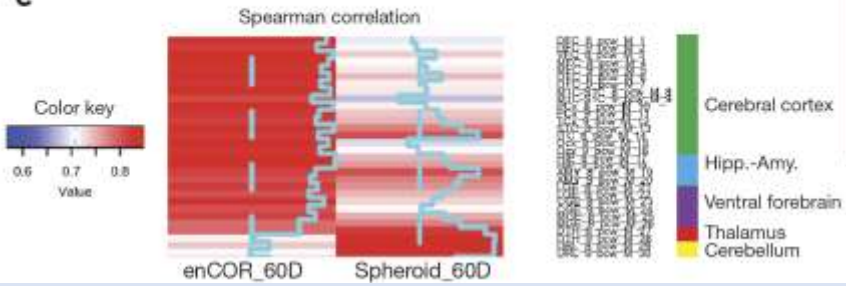
Nat Biotech 2017

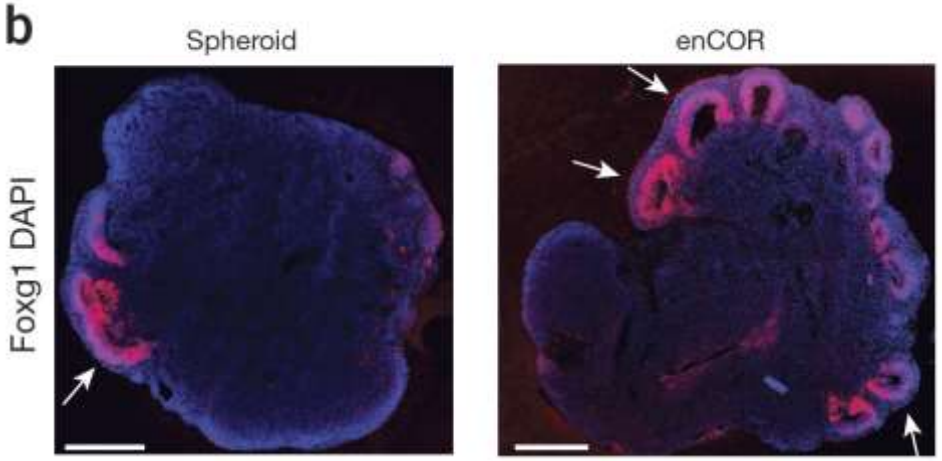
Madeline A Lancaster[1,2], Nina S Corsini[1], Simone Wolfinger[1], E Hilary Gustafson[1], Alex W Phillips[2], Thomas R Burkard[1,3], Tomoki Otani[4], Frederick J Livesey[4] & Juergen A Knoblich[1]











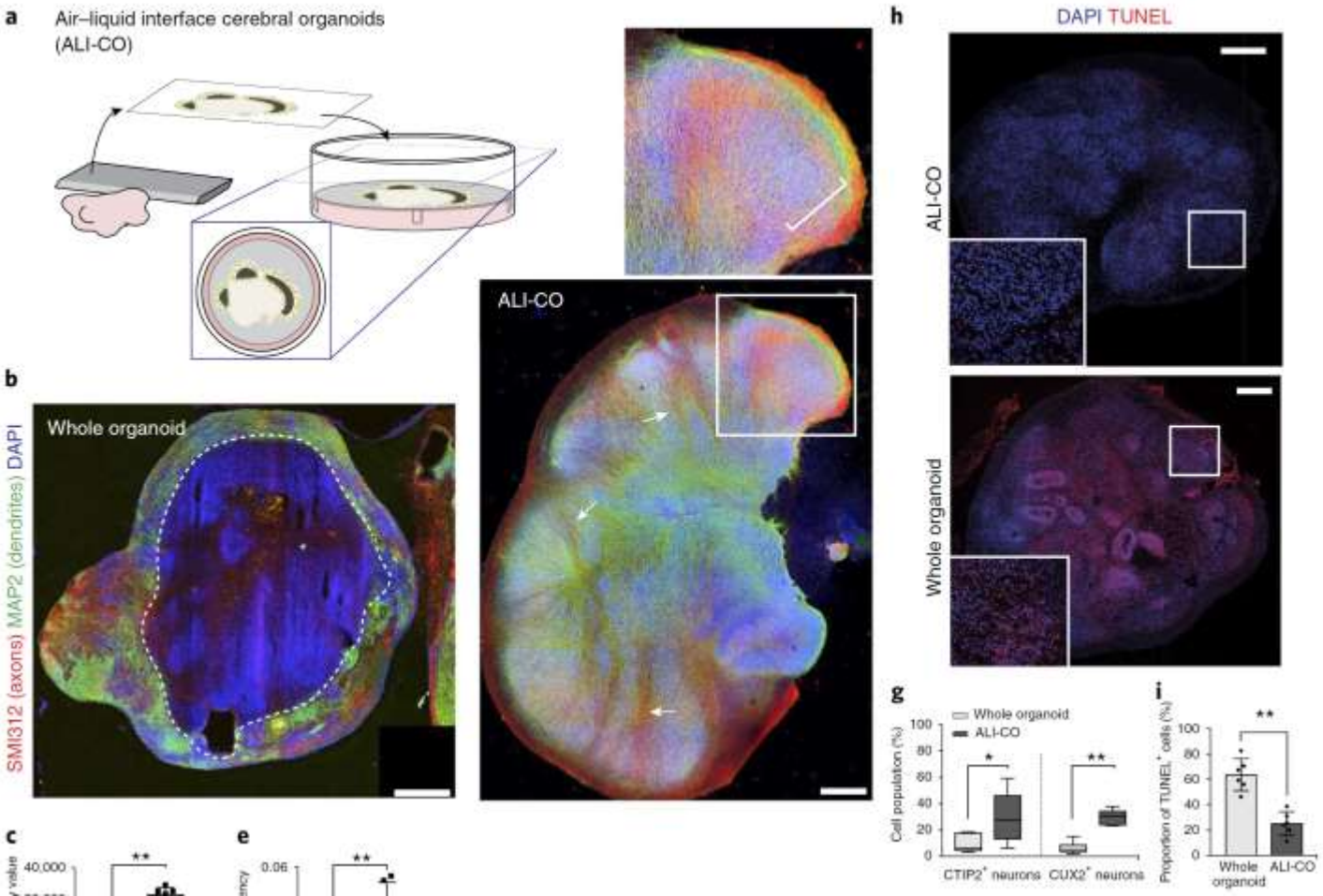
enCORs show increased forebrain identity. (**b**) Representative sections of whole 40-d H9 **organoids stained for the forebrain marker Foxg1**. enCORs display more Foxg1+ lobes (arrows) compared with spheroids. (**c**) **Quantification** of the mean ratio of individual lobes displaying positive staining **for the specified regional markers**. Foxg1+ regions represent forebrain, regions highly positive for Otx2 represent midbrain, En2+ regions represent cerebellar or hindbrain identities. Error bars are s.e.m. **P* < 0.01, ***P* < 0.0001, Student's two-tailed *t*-test, *n* = 8 spheroids (40 d, H9) from three independent batches, *n* = 11 enCOR organoids (40 d, H9) from four independent batches. (**d**) Staining of day 40 H9 enCOR brain organoids and spheroids for the markers of **dorsal cortex Tbr1 and Tbr2** reveals large lobes of tissue that are dorsal cortex (arrows) in enCORs. Spheroids show many fewer dorsal regions and some large brain regions that lack this identity (arrowhead). (**e**) Heatmap of **Spearman correlation coefficients of differentially expressed genes** at 60 d in H9 **spheroids and enCORs with the Allen BrainSpan transcriptome**. All brain regions are shown for stage I (8–9 post-conception weeks), sorted by anterior-posterior regional identity. Hipp.-Amy.: Hippocampus – Amygdala. Scale bars: 500 mm in **b**,**d**.

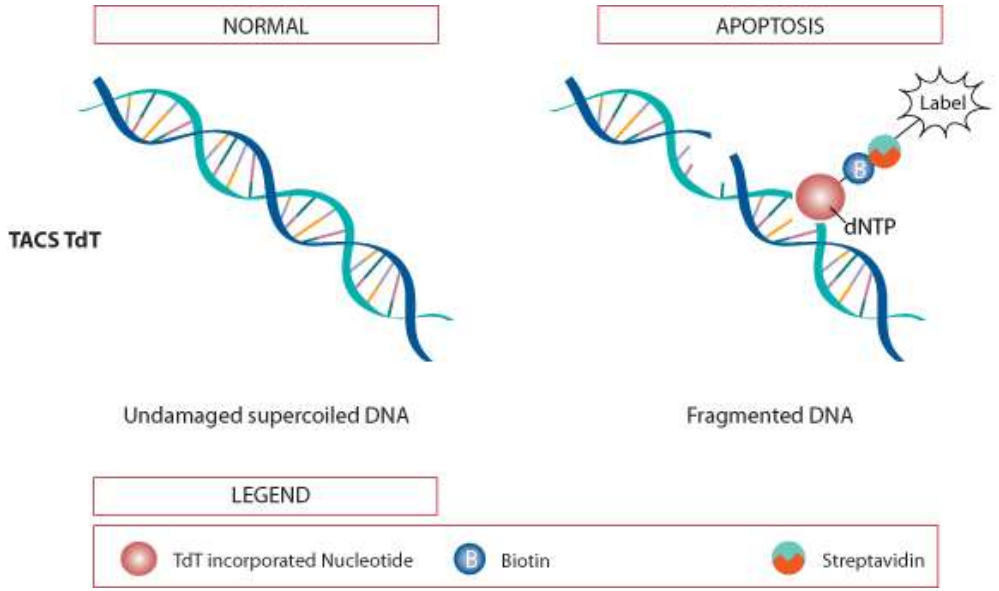
Other strategies for culturing brain organoids

Cerebral organoids at the air–liquid interface generate diverse nerve tracts with functional output

Nature Neuroscience 2019

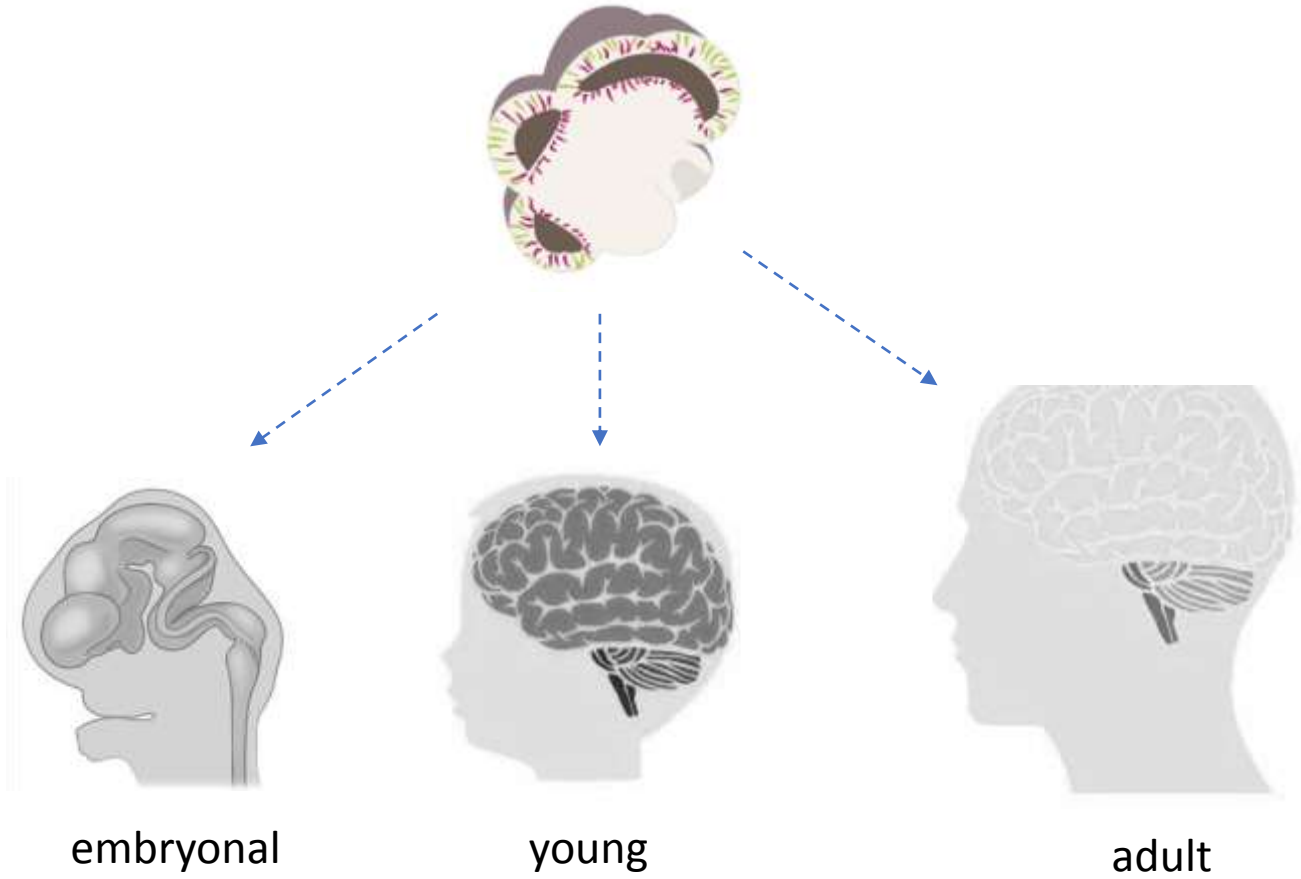
Stefano L. Giandomenico[1], Susanna B. Mierau[2], George M. Gibbons[3], Lea M. D. Wenger[3], Laura Masullo[1], Timothy Sit[2], Magdalena Sutcliffe[1], Jerome Boulanger[1], Marco Tripodi[1], Emmanuel Derivery[1], Ole Paulsen[2], András Lakatos[3,4] and Madeline A. Lancaster[1*]





DNA fragmentation represents a characteristic hallmark of apoptosis. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) is an established method for detecting DNA fragments.

Cerebral organoids:
How relevant for modeling nervous tissue architecture and stage?

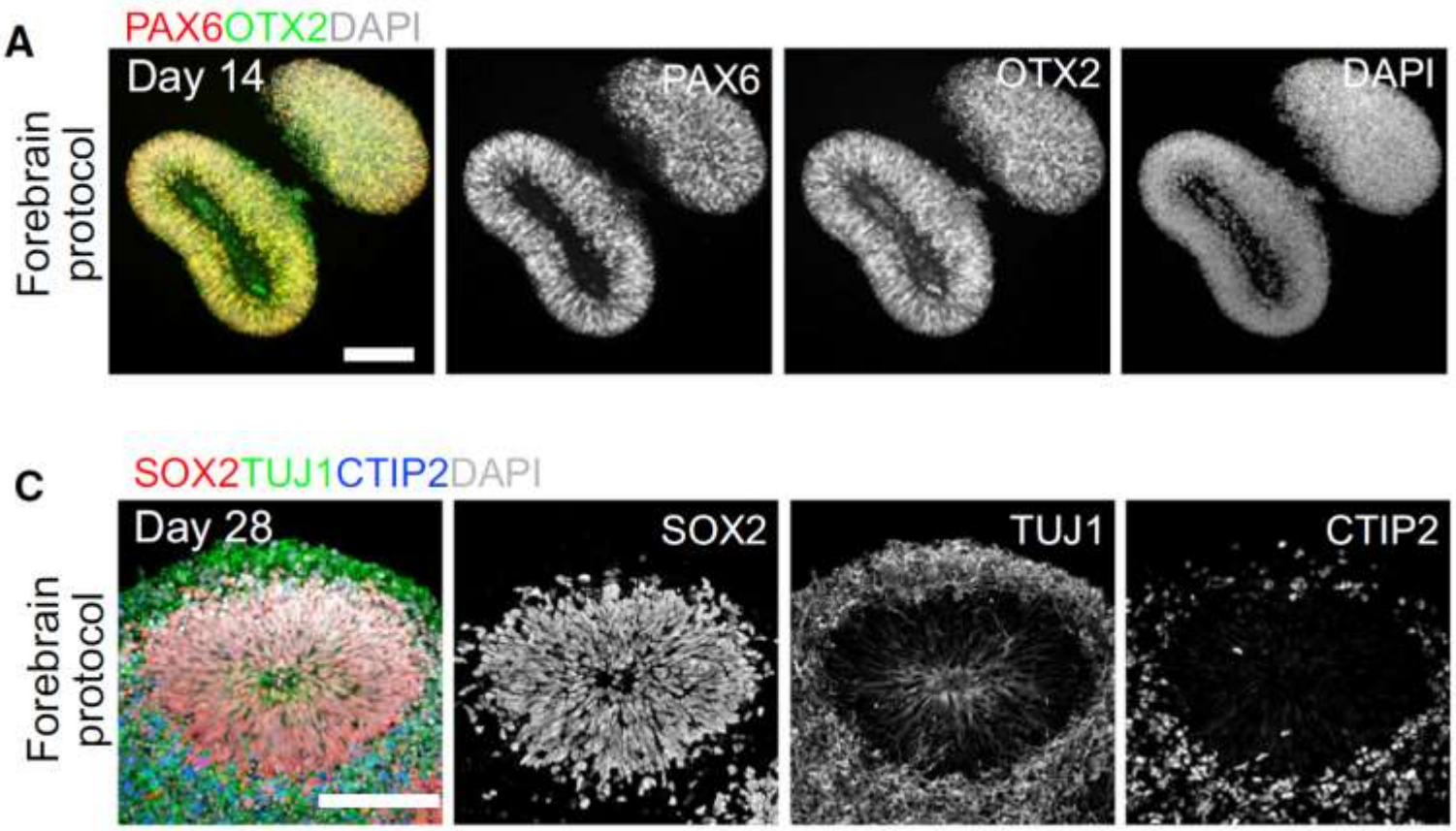


Cerebral organoids: How relevant for modeling nervous tissue architecture and stage?

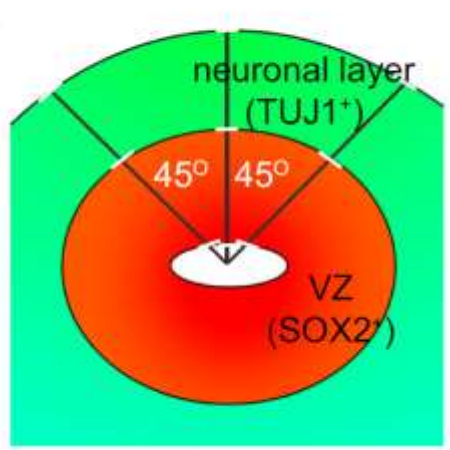
May 2016

Cell

Brain-Region-Specific Organoids Using Mini-bioreactors for Modeling ZIKV Exposure



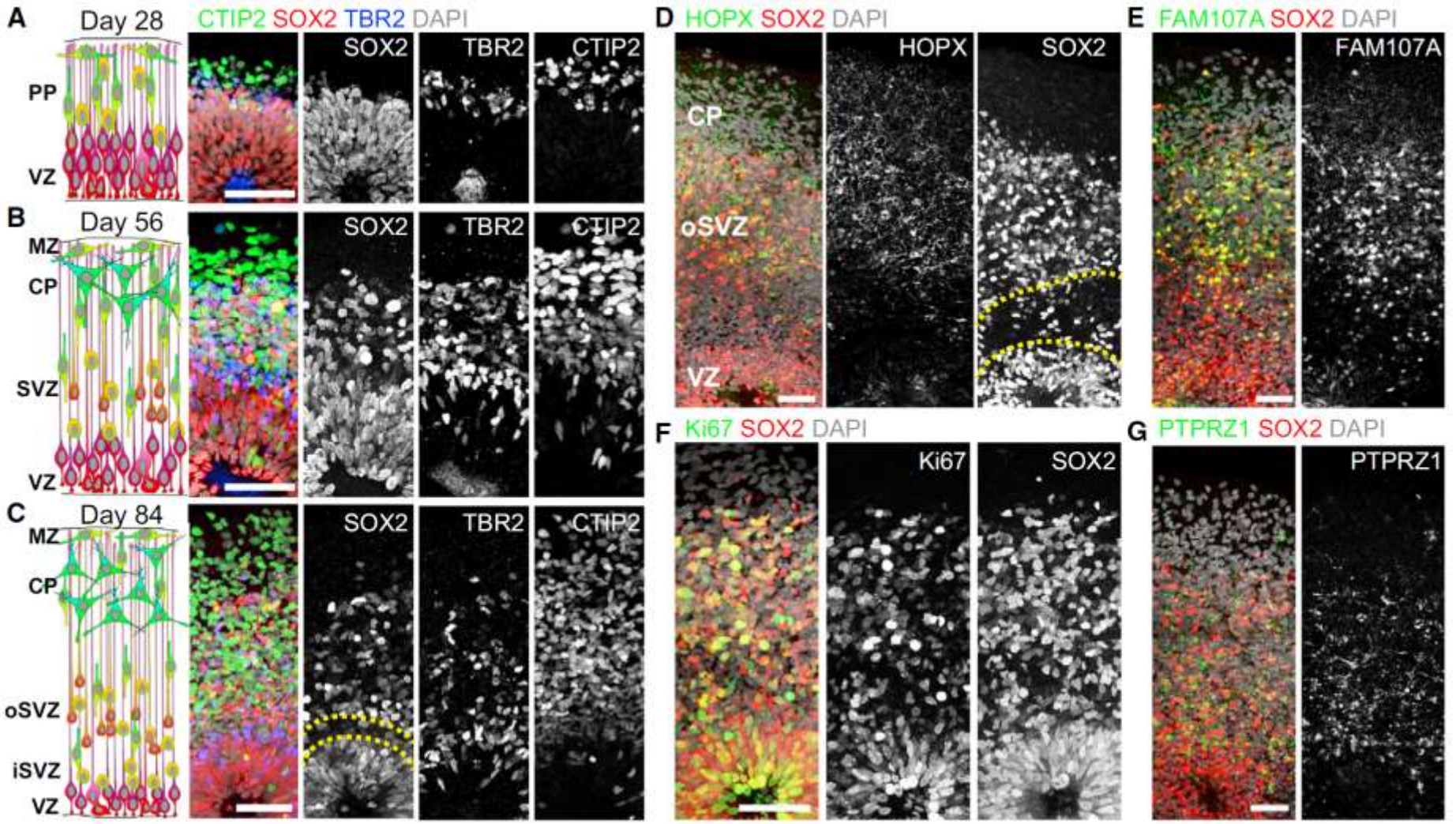
Organoids present cell structures disposed in a radial organization, composed by a ventricular zone-like (VZ) layer and TUJ1+ neuronal layer between apical and basal surfaces organization established over time.

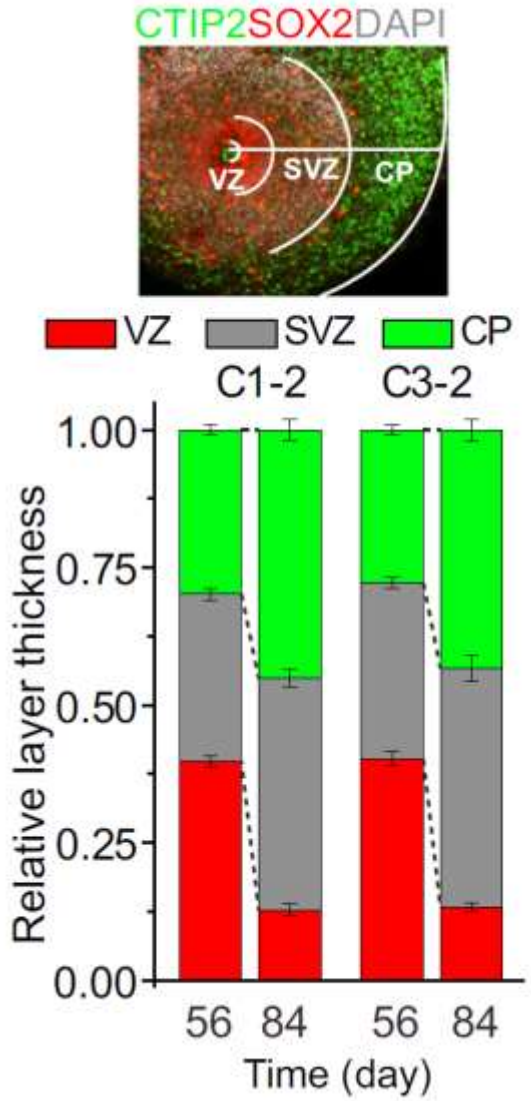


Cerebral organoids: How relevant for modeling nervous tissue architecture and stage?

Cell May 2016

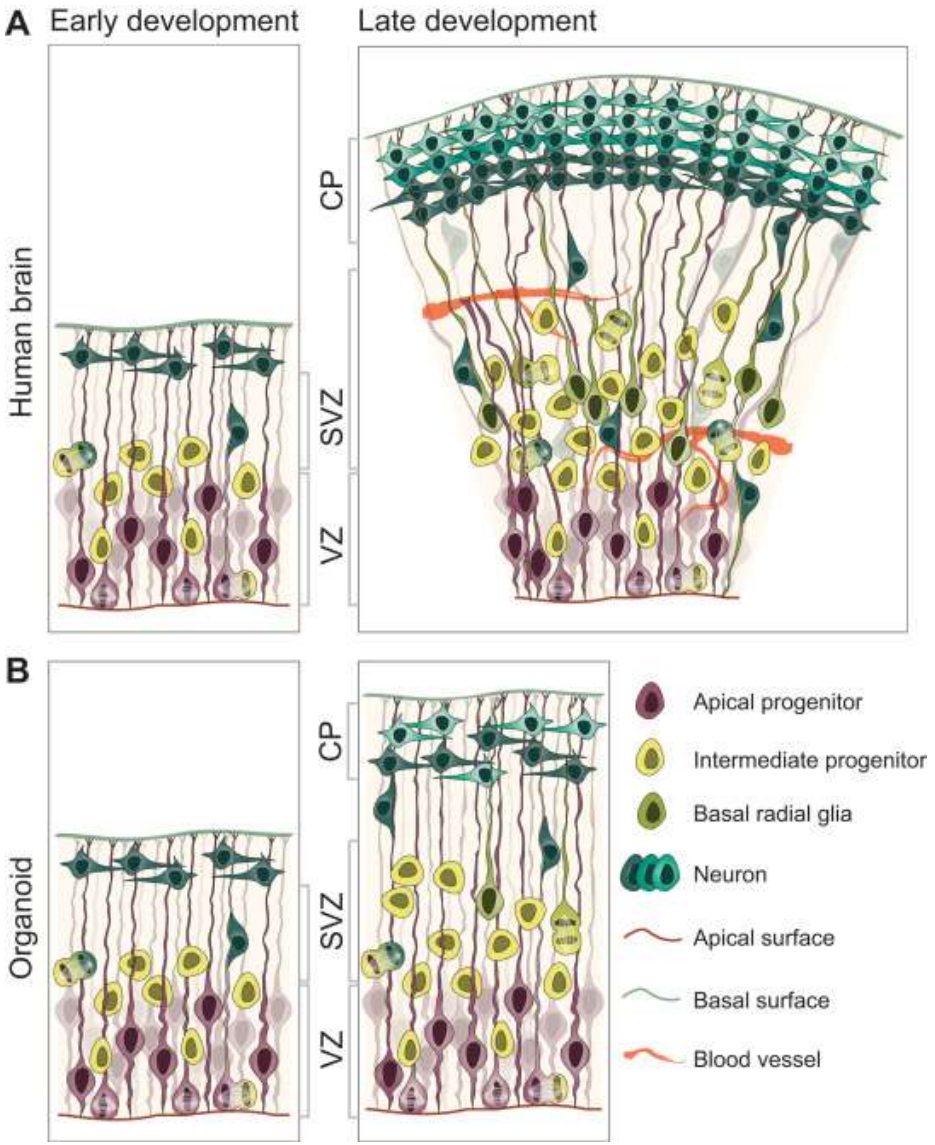
Brain-Region-Specific Organoids Using Mini-bioreactors for Modeling ZIKV Exposure





Cerebral organoids: How relevant for modeling nervous tissue architecture and stage?

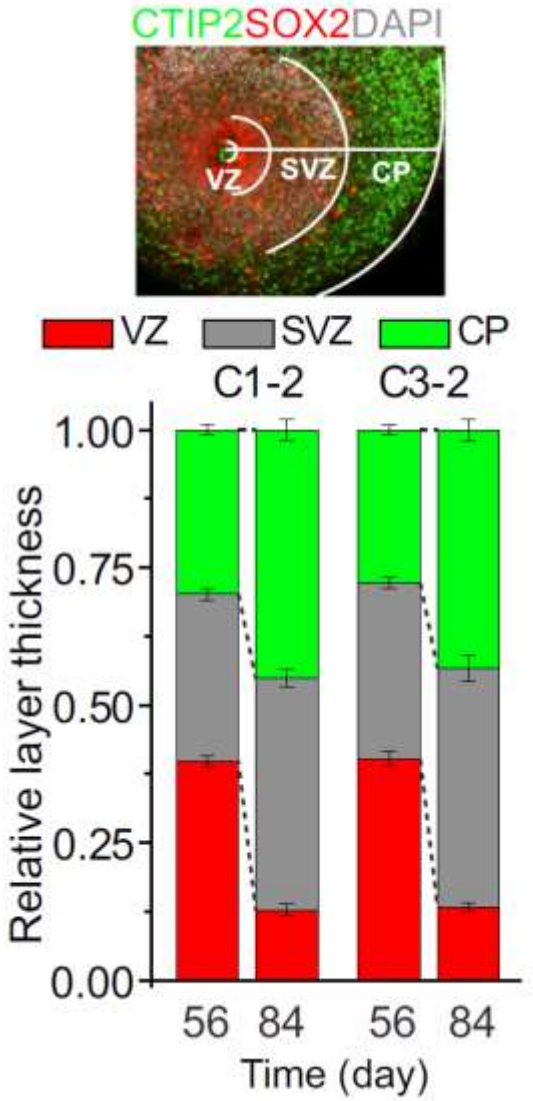
Comparison of in vivo and in vitro brain development. A simplified representation of the cell biological complexity of the in vivo developing brain and the in vitro brain organoid. ***The early stages (left) possess a similar morphological level of complexity***. ***Later stages*** (right) ***differ in the size of the cortical wall and diversity and complexity of neural progenitor populations.*** Note the absence of vasculature (orange) in the organoid, a reduced SVZ and the rudimentary organization of the neuronal layers. VZ – ventricular zone,SVZ – subventricularzone, CP – cortical plate.



Kelava et al; Dev Biol 2016

Cell *May 2016*

Brain-Region-Specific Organoids Using Mini-bioreactors for Modeling ZIKV Exposure



Cerebral organoids: How relevant for modeling nervous architecture and stage?

July 2014

Neuron
NeuroResource

CORTECON: A Temporal Transcriptome Analysis of In Vitro Human Cerebral Cortex Development from Human Embryonic Stem Cells

Joyce van de Leemput,[1,4] Nathan C. Boles,[1,4] Thomas R. Kiehl,[1,2] Barbara Corneo,[1] Patty Lederman,[1] Vilas Menon,[3] Changkyu Lee,[3] Refugio A. Martinez,[3] Boaz P. Levi,[3] Carol L. Thompson,[3] Shuyuan Yao,[3] Ajamete Kaykas,[3] Sally Temple,[1,5,*] and Christopher A. Fasano[1,5,*]

In-vitro 2D human cerebral cortex protocol

Cell *July 2015*

FOXG1-Dependent Dysregulation of GABA/Glutamate Neuron Differentiation in Autism Spectrum Disorders

Jessica Mariani,[1,2,9] Gianfilippo Coppola,[1,2,9] Ping Zhang,[3] Alexej Abyzov,[1,4,10] Lauren Provini,[1,2] Livia Tomasini,[1,2] Mariangela Amenduni,[1,2] Anna Szekely,[1,5] Dean Palejev,[1,2,11] Michael Wilson,[1,2] Mark Gerstein,[1,4,6,7] Elena L. Grigorenko,[1,2] Katarzyna Chawarska,[1,2] Kevin A. Pelphrey,[1,2] James R. Howe,[3] and Flora M. Vaccarino[1,2,8,*]

Telencephalic organoids (iPSCs from macrocephaly ASD patients)

nature biotechnology *May 2017*

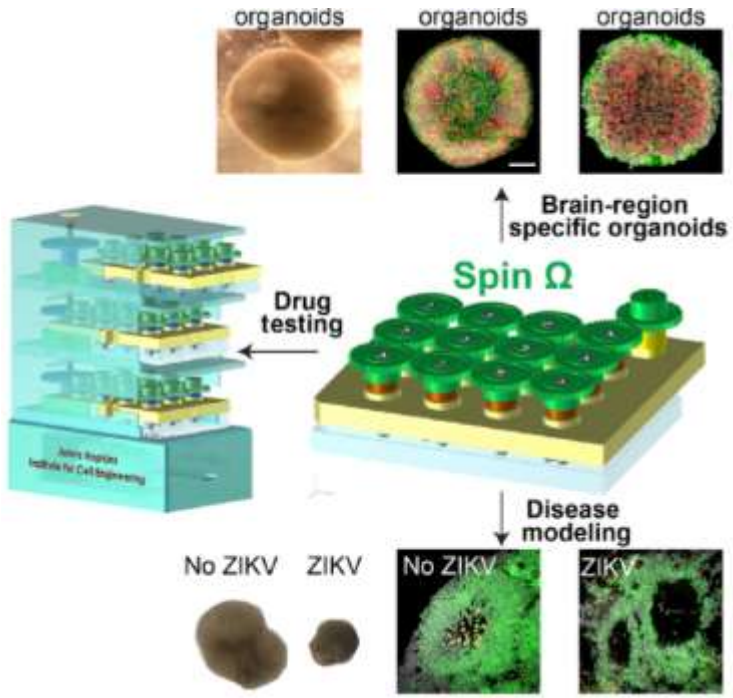
Guided self-organization and cortical plate formation in human brain organoids

Madeline A Lancaster[1,2], Nina S Corsini[1], Simone Wolfinger[1], E Hilary Gustafson[1], Alex W Phillips[2], Thomas R Burkard[1,3], Tomoki Otani[4], Frederick J Livesey[4] & Juergen A Knoblich[1]

Cell Resource *May 2016*

Brain-Region-Specific Organoids Using Mini-bioreactors for Modeling ZIKV Exposure

Xuyu Qian,[1,2,13] Ha Nam Nguyen,[1,2,3,4,13] Mingxi M. Song,[1,9] Christopher Hadiono,[1,10] Sarah C. Ogden,[11] Christy Hammack,[11] Bing Yao,[12] Gregory R. Hamersky,[5] Fadi Jacob,[1] Chun Zhong,[1,4] Ki-jun Yoon,[1,4] William Jeang,[1,14] Li Lin,[12] Yujing Li,[12] Jai Thakor,[1] Daniel A. Berg,[1] Ce Zhang,[1,4] Eunchai Kang,[1,4] Michael Chickering,[1] David Nauen,[1,6] Cheng-Ying Ho,[15,16] Zhexing Wen,[1,4] Kimberly M. Christian,[1,4] Pei-Yong Shi,[17] Brady J. Maher,[5,7] Hao Wu,[13] Peng Jin,[12] Hengli Tang,[11] Hongjun Song,[1,2,4,6,*] and Guo-li Ming[1,2,3,4,7,8,*]

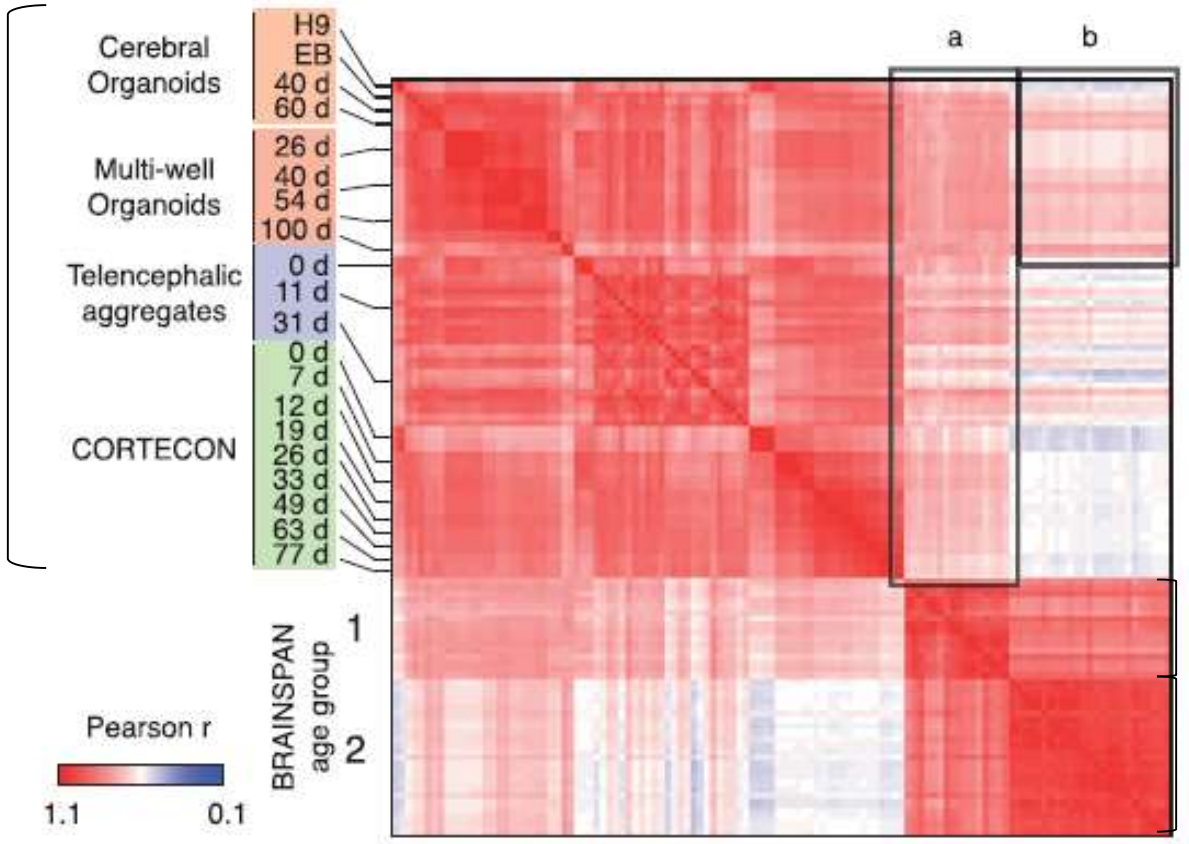


Cerebral organoids

Chongyuan Luo,[1,2,4] Madeline A. Lancaster,[3,4,5] Rosa Castanon,[1] Joseph R. Nery,[1] Juergen A. Knoblich,[3,*] and Joseph R. Ecker[1,2,6,*]
[1]Genomic Analysis Laboratory, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA
[2]Howard Hughes Medical Institute, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA
[3]Institute of Molecular Biotechnology of the Austrian Academy of Science (IMBA), Vienna 1030, Austria
[4]Co-first author
[5]Present address: MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, UK
[6]Lead Contact

Cell Reports 2016

In-vitro systems



Cerebral organoids (Lancaster protocol), and "multi-well organoids (Qian's mini-bioreactor approach) generated tissues with transcriptome profiles similar to those observed in early and mid-stage human brain developmental samples (60 to 100 days of culture).

Cortex samples

Early (8-9 PCW)

Mid (12-16 PCW)

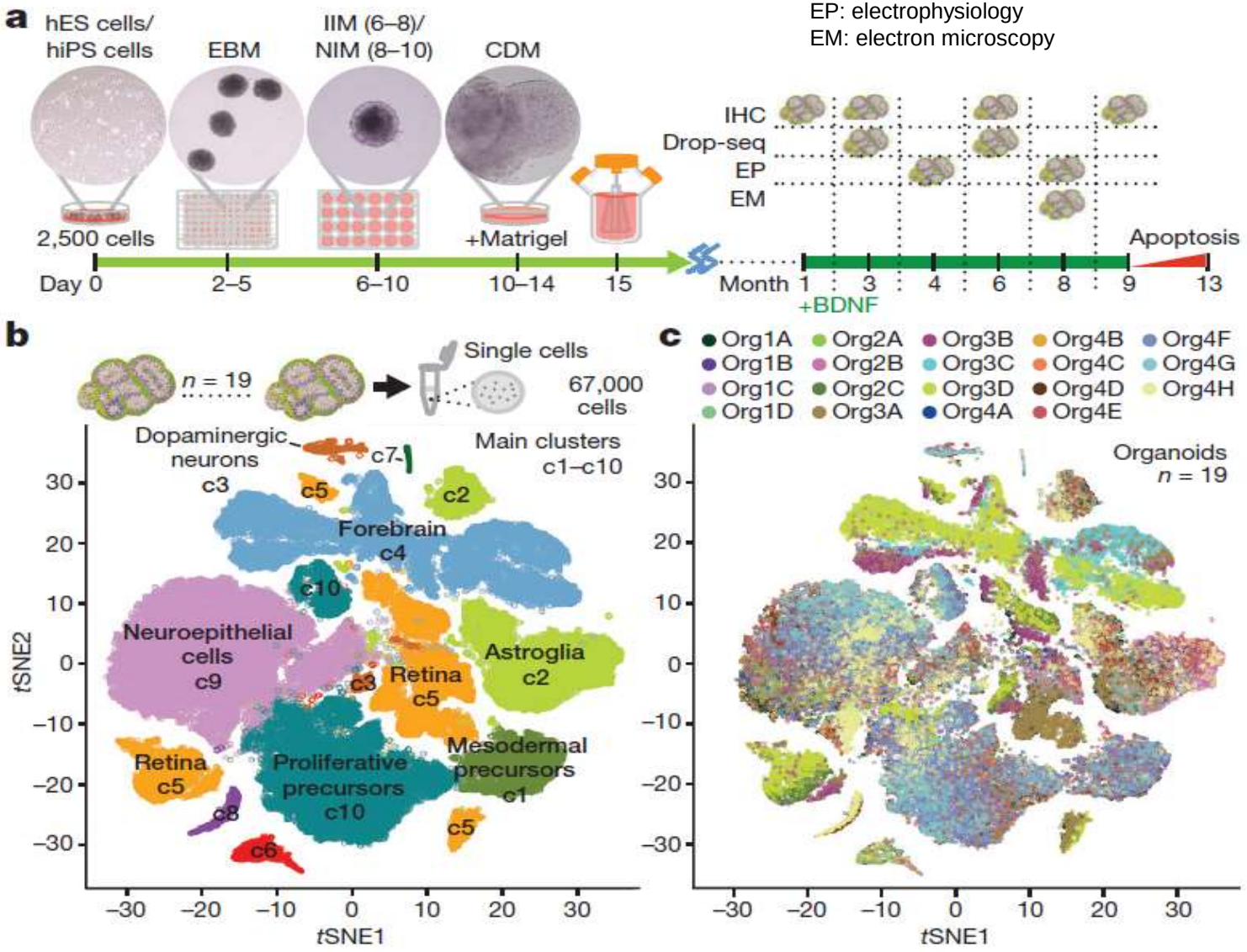
Humain brain developmental transcriptome (**RNA-seq**)

- H9: human ESC (hESC H9)
- EB: Embryoid body (prior vitA induction)

PCW:post-conceptional weeks

Cerebral organoids

IHC: immunohistochemistry
Drop-seq: single-cell RNA-seq
EP: electrophysiology
EM: electron microscopy

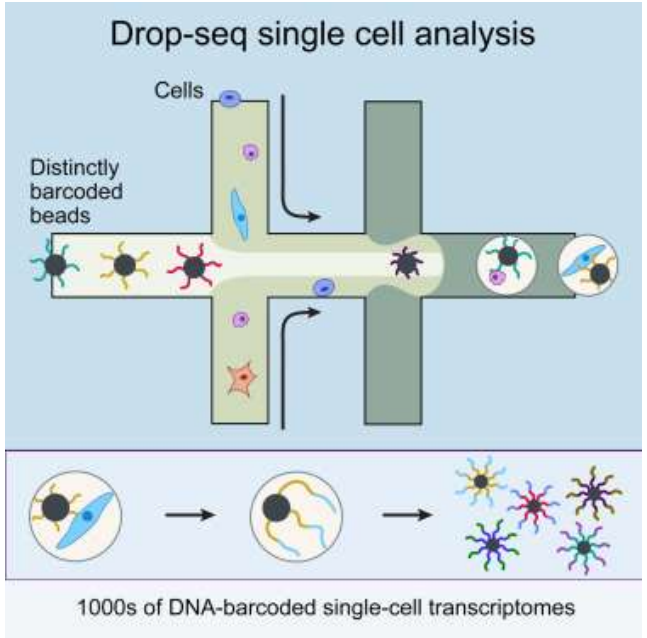


ARTICLE
48 | NATURE | VOL 545 | 4 MAY 2017

Cell diversity and network dynamics in photosensitive human brain organoids

Giorgia Quadrato[1,2], Tuan Nguyen[1,2], Evan Z. Macosko[2,3], John L. Sherwood[1,2], Sung Min Yang[2], Daniel R. Berger[4], Natalie Maria[1], Joeg Scholvin[5], Melissa Goldman[3], Justin P. Kinney[6], Edward S. Boyden[5], Jeff W. Lichtman[4], Ziv M. Williams[7], Steven A. McCarroll[2,3] & Paola Arlotta[1,2]

6 months brain organoids cultures analyzed by single-cell RNA-sequencing (Drop-seq) demonstrated a major cell diversity

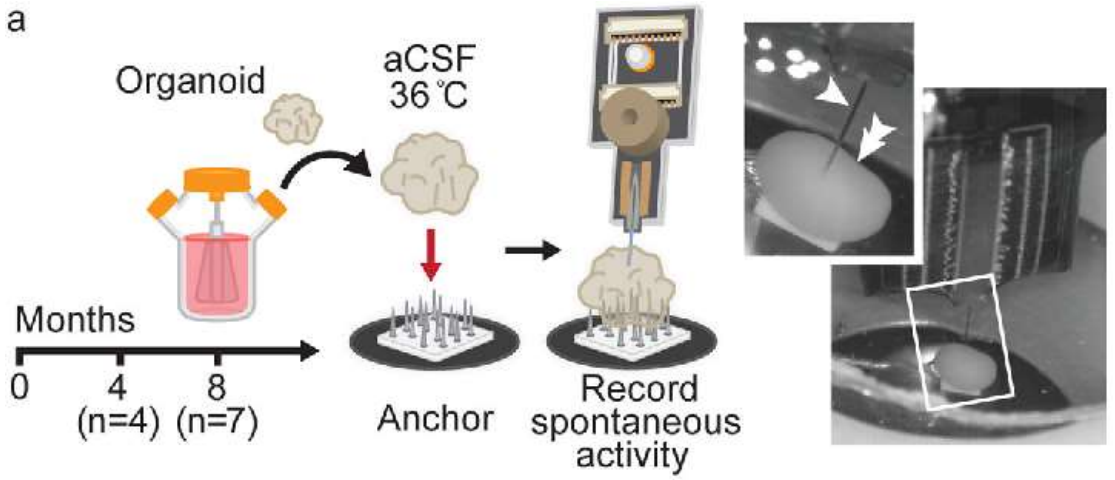


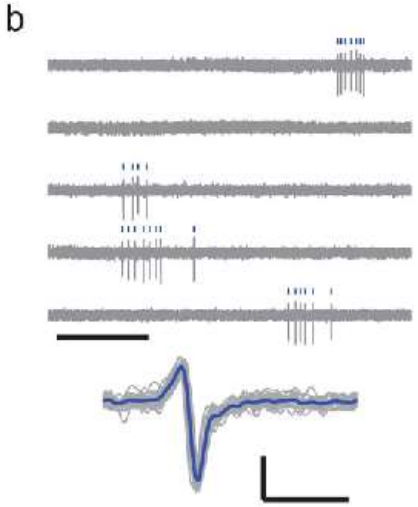
Cerebral organoids

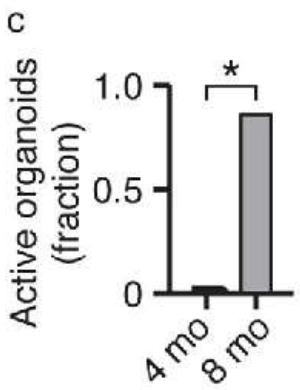
ARTICLE 48 | NATURE | VOL 545 | 4 MAY 2017

Cell diversity and network dynamics in photosensitive human brain organoids

Giorgia Quadrato[1,2], Tuan Nguyen[1,2], Evan Z. Macosko[2,3], John L. Sherwood[1,2], Sung Min Yang[2], Daniel R. Berger[4], Natalie Maria[1], Joerg Scholvin[5], Melissa Goldman[1], Justin P. Kinney[6], Edward S. Boyden[5], Jeff W. Lichtman[4], Ziv M. Williams[2], Steven A. McCarroll[2,3] & Paola Arlotta[1,2]



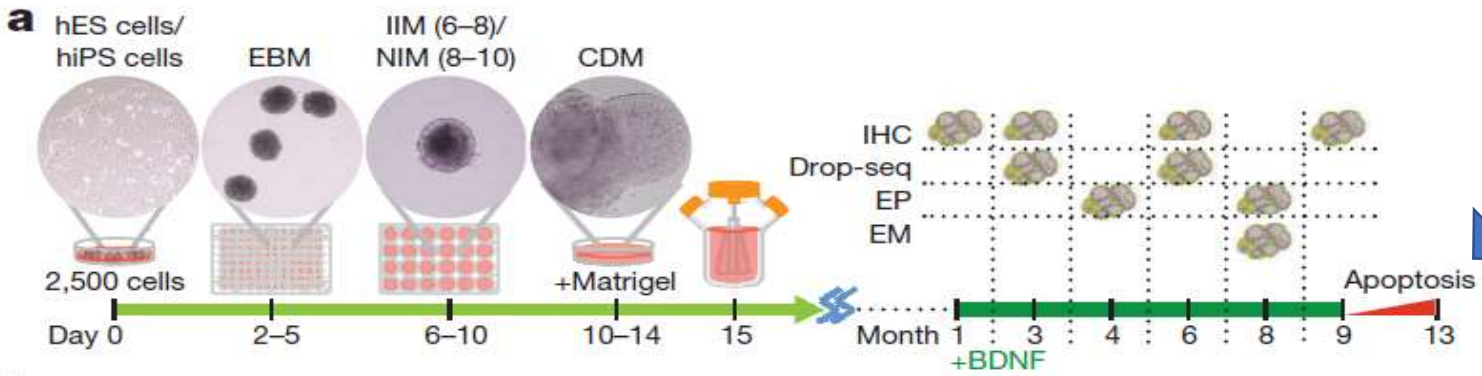




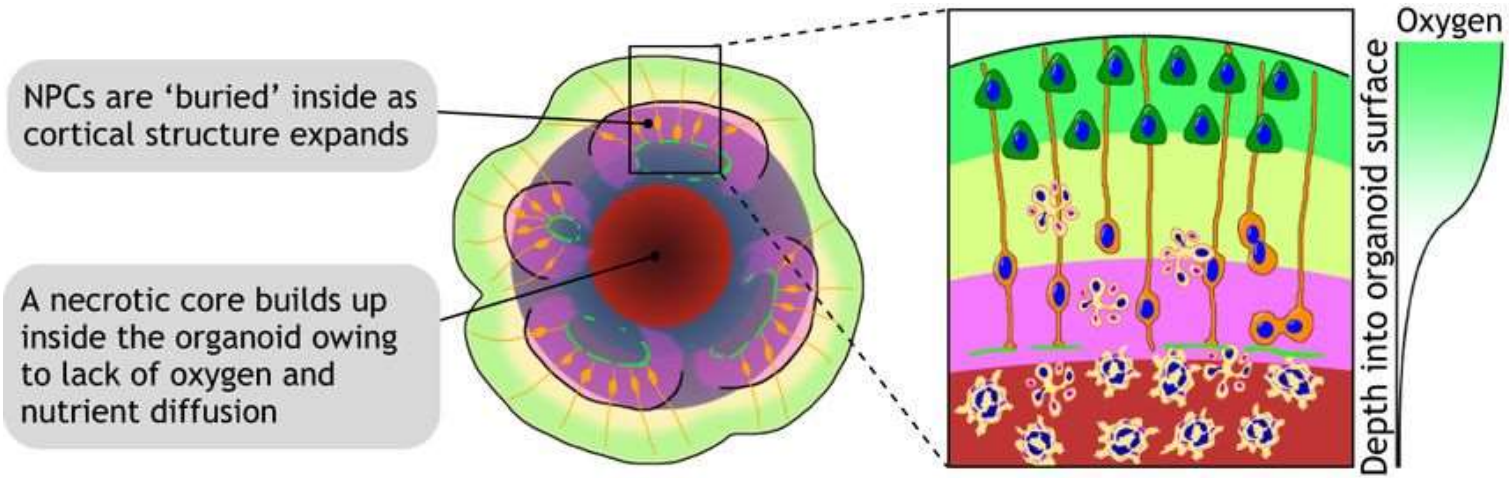
Brain organoids develop spontaneous electric activity

a. Schematic and photograph of extracellular recordings from intact organoids. (arrow, probe; double arrow, organoid). **b.** Human brain organoids display spontaneous activity. Top, example raw traces and spike raster plots from a single unit (scale bar 0.5 s). Bottom, individual (gray) and average (blue) spike waveforms (scale bars, 0.5 ms, 50 μV). **c.** Organoids display spontaneous activity at 8 months (6 of 7) but not 4 months (0 of 4; Fisher's exact test, p=0.015).

Cerebral organoids



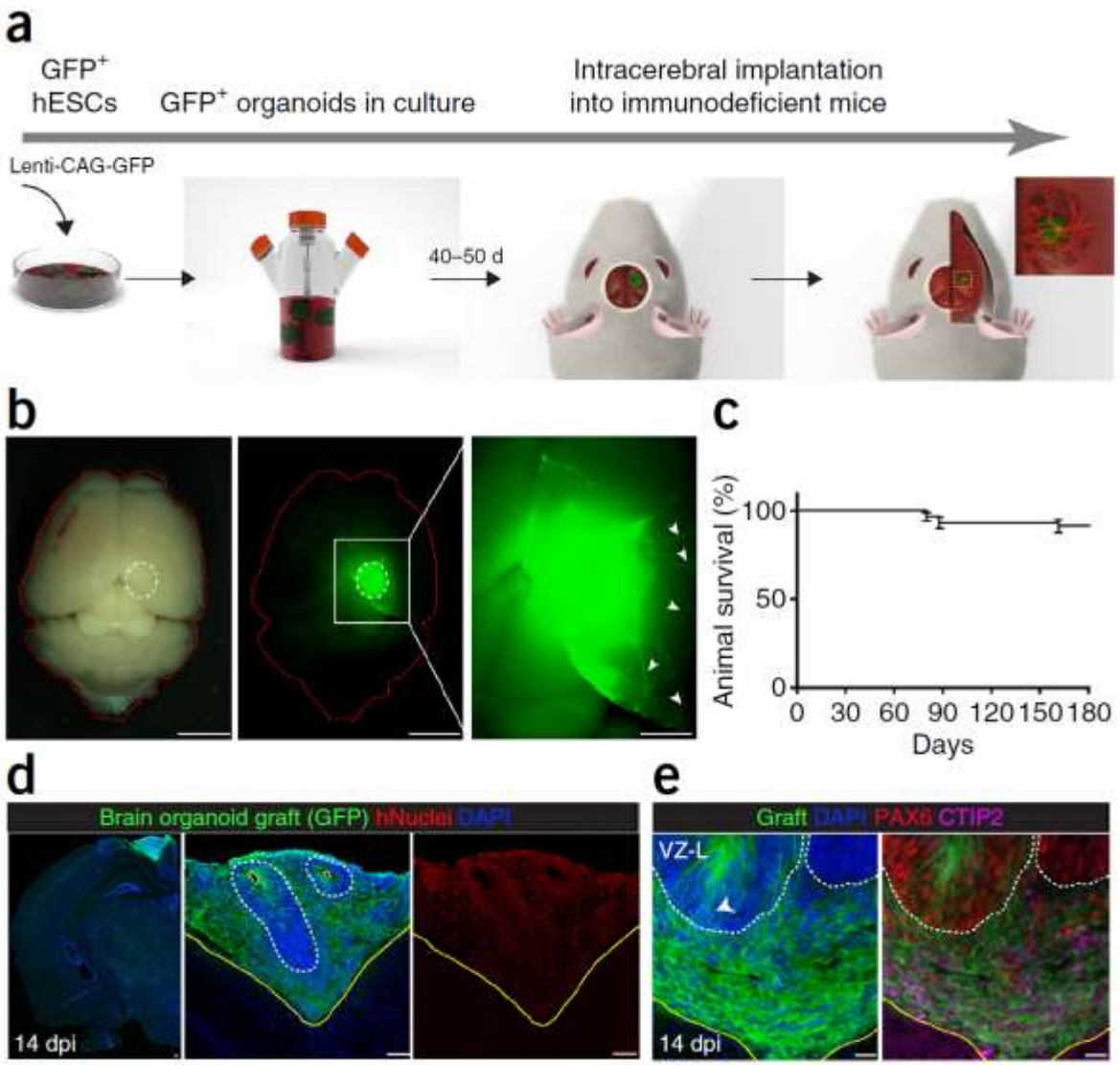
Apoptosis/necrosis has been reported during brain organoids progression. This issue limits culturing these type of 3d-tissues over even longer periods.



- Cells near the organoid surface receive sufficient oxygen and nutrients
- Oxygen becomes limited deeper into the surface
- Acidification and released inflammatory contents induce apoptosis of neighboring cells; adherens junctions at the apical surface are disrupted
- Cell necrosis occurs due to lack of oxygen and nutrients

Qian et al; Development 2019

Cerebral organoids



An *in vivo* model of functional and vascularized human brain organoids

Nat Biotech 2017

Abed AlFatah Mansour[1], J Tiago Gonçalves[1,4], Cooper W Bloyd[1], Hao Li[2], Sarah Fernandes[1,3], Daphne Quang[1], Stephen Johnston[1], Sarah L Parylak[1], Xin Jin[2] & Fred H Gage[1]

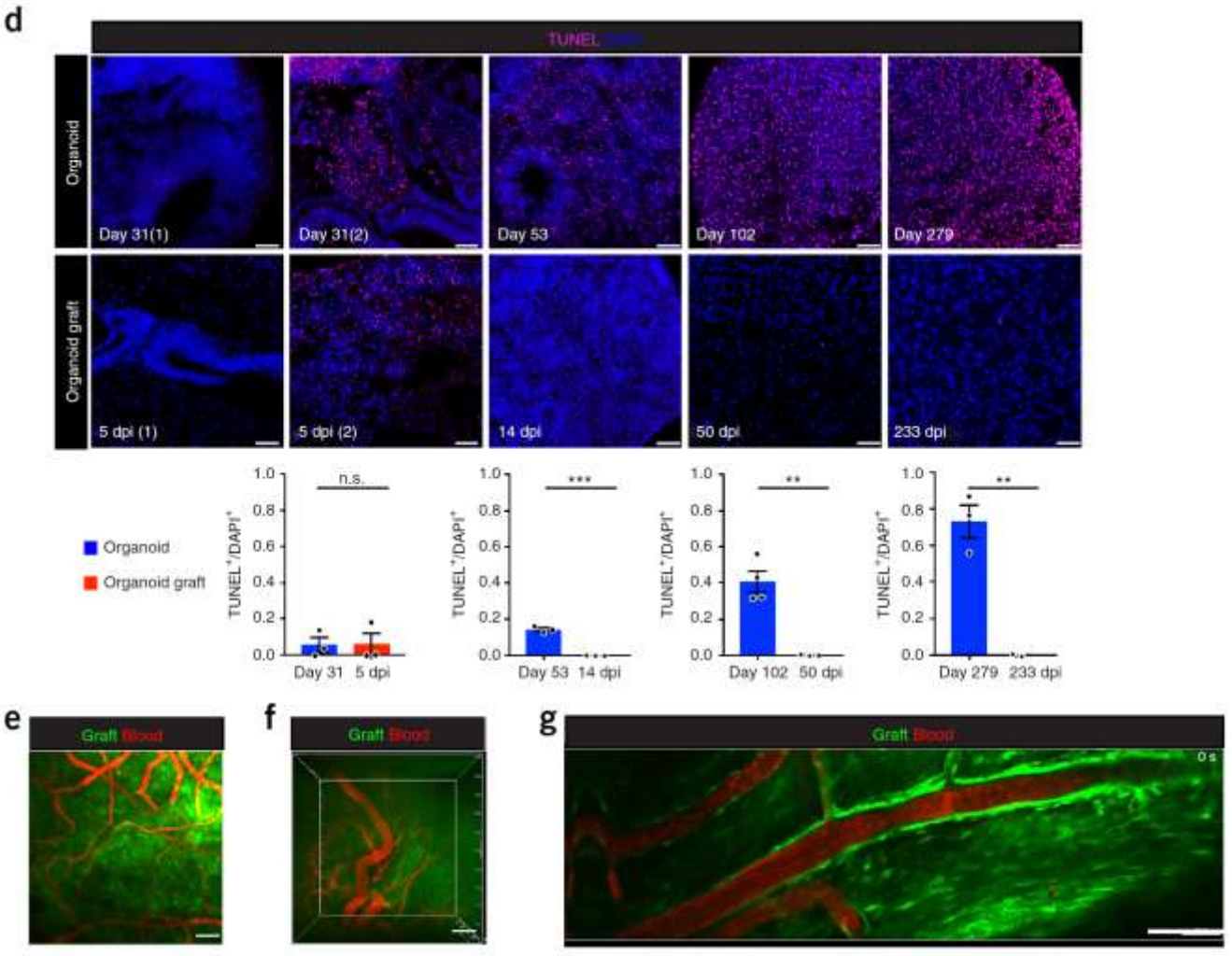
Intracerebral grafting of brain organoids into mouse brain.
(a) Illustration of the experimental procedure for generation of GFP+ organoids from hESCs and intracerebral implantation into immunodeficient mouse brain. **(b)** Whole-mount dorsal view image of mouse brain grafted with a GFP+ cerebral organoid and harvested at 50 dpi; the graft is outlined in white. ***Right, magnified graft displaying neurite outgrowth from the organoid toward the host brain (arrowheads).***
(c) Kaplan–Meier survival curve for overall survival of mice after engraftment with brain organoids. (91.8% survival beyond 180 dpi, *n* = 61 mice from ten experiments). **(d)** GFP+ organoids were grafted into mouse brain and harvested at the indicated dpi. Coronal sections were analyzed using immunofluorescence and confocal microscopy. Immunofluorescence staining for GFP and human nuclear antigen (hNuclei), demonstrates that the implant survived well and distributed throughout the lesion cavity at 14 dpi. Left image shows confocal stitched tile scan; image was vertically inverted. White solid lines indicate apical/ventricular surface. Dotted white line indicates the radial glia VZ-L regions. Yellow lines indicate the graft-host border. **(e)** Graft immunostained for GFP, dorsal telencephalic progenitor marker PAX6 and the deep-layer subcortical neuron marker CTIP2. Radially organized cells (arrowhead, left panel) represent the PAX6+ VZ-L region (dotted white lines). *n* = 4 animals in b, and *n* = 3 animals in d,e. Nuclei were counterstained with DAPI. Scale bars: 1 mm in b, 100 µm in d, and 20 µm in e.

Cerebral organoids

An *in vivo* model of functional and vascularized human brain organoids

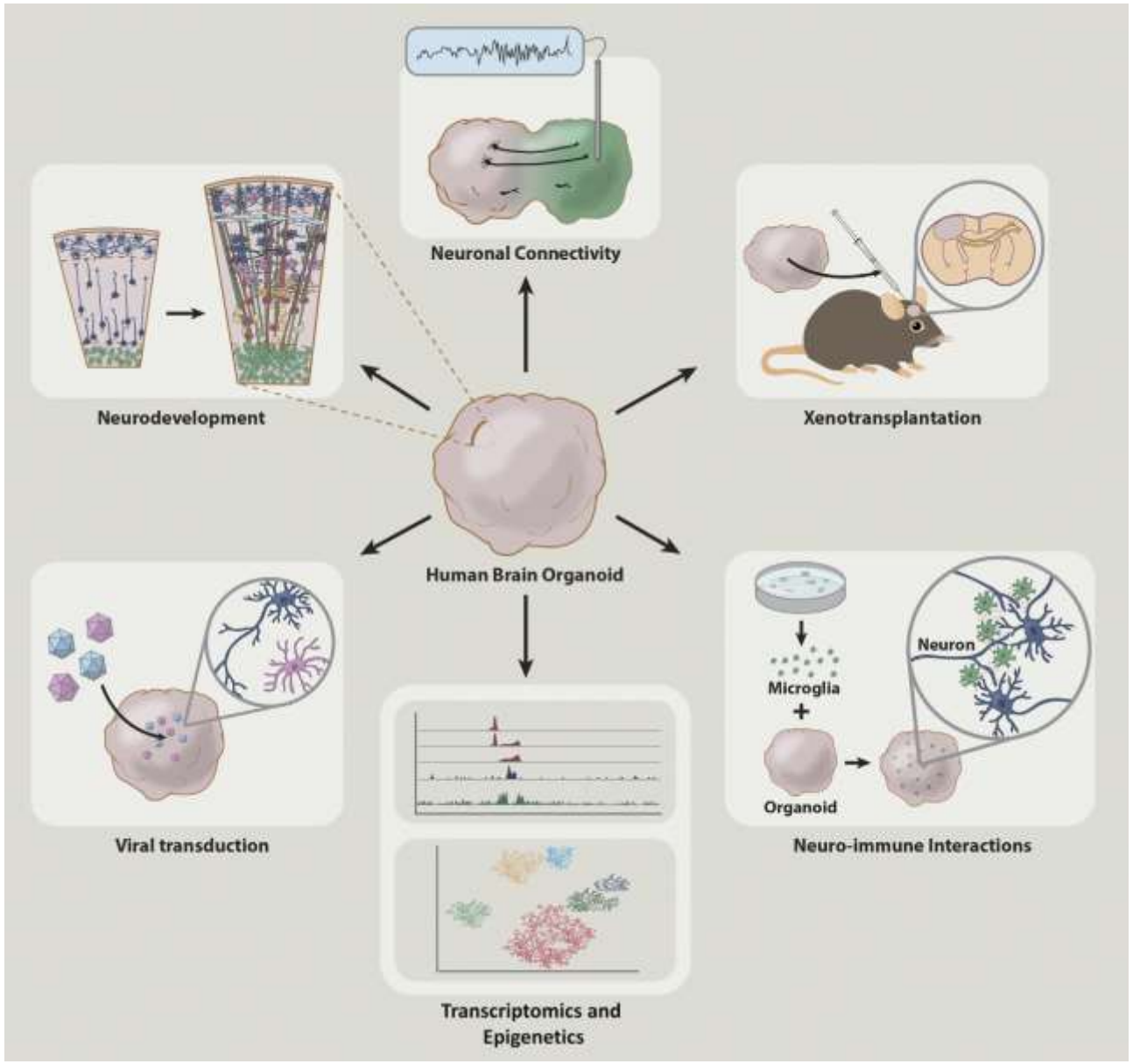
Nat Biotech 2017

Abed AlFatah Mansour[1], J Tiago Gonçalves[1,4], Cooper W Bloyd[1], Hao Li[2], Sarah Fernandes[1,3], Daphne Quang[1], Stephen Johnston[1], Sarah L Parylak[1], Xin Jin[2] & Fred H Gage[1]



Decreased apoptosis in grafts. (d) Organoids have an elevated degree of cell death that is rescued after grafting. Top, TUNEL staining of grafted organoids and stage-matched cultured organoids at the indicated stage. Left panels show staining obtained from two different organoids and organoid grafts at day 31 and 5 dpi, respectively. Bottom, quantification of TUNEL+/DAPI+ cells in grafted organoids and nearly stage-matched cultured organoids of indicated ages. Values are represented as mean ± s.e.m., (n = 3, except for 102-d organoid, n = 4, and 233 dpi n = 2); unpaired two-tailed t-test was used to compare mean difference between each group. Day 31 vs. 5 dpi (t = 0.03656, df = 4, P = 0.9726, not significant), day 53 vs. 14 dpi (t = 14.67, df = 4, P = 0.0001), day 102 vs. 50 dpi (t = 5.943, df = 5, P = 0.0019), and day 279 vs. 233 dpi (t = 6.267, df = 3, P = 0.0082). **(e–g)** *In vivo* two-photon imaging of blood vessels via dextran infusion as viewed through the cranial window. Organoids were implanted and TexasRed-dextran was injected at different time points of postimplantation (30 dpi in e, 120 dpi in f). **(e)** maximum projection of a 300-µm stack taken in a 30-dpi graft. **(f)** top view of a three-dimensional reconstruction of a 500-µm Z section in the organoids from a 120-dpi grafted animal. **(g)** Single z-plane obtained from 120-dpi graft and acquired at 141-µm depth below the organoid surface, showing blood flow in the vasculature network. Nuclei were counterstained with DAPI. Scale bars: 1 mm in b, 50 µm in c,d, and 100 µm in e–g. $^{**}P < 0.01$, $^{***}P < 0.001$.

Cerebral organoids: Applications

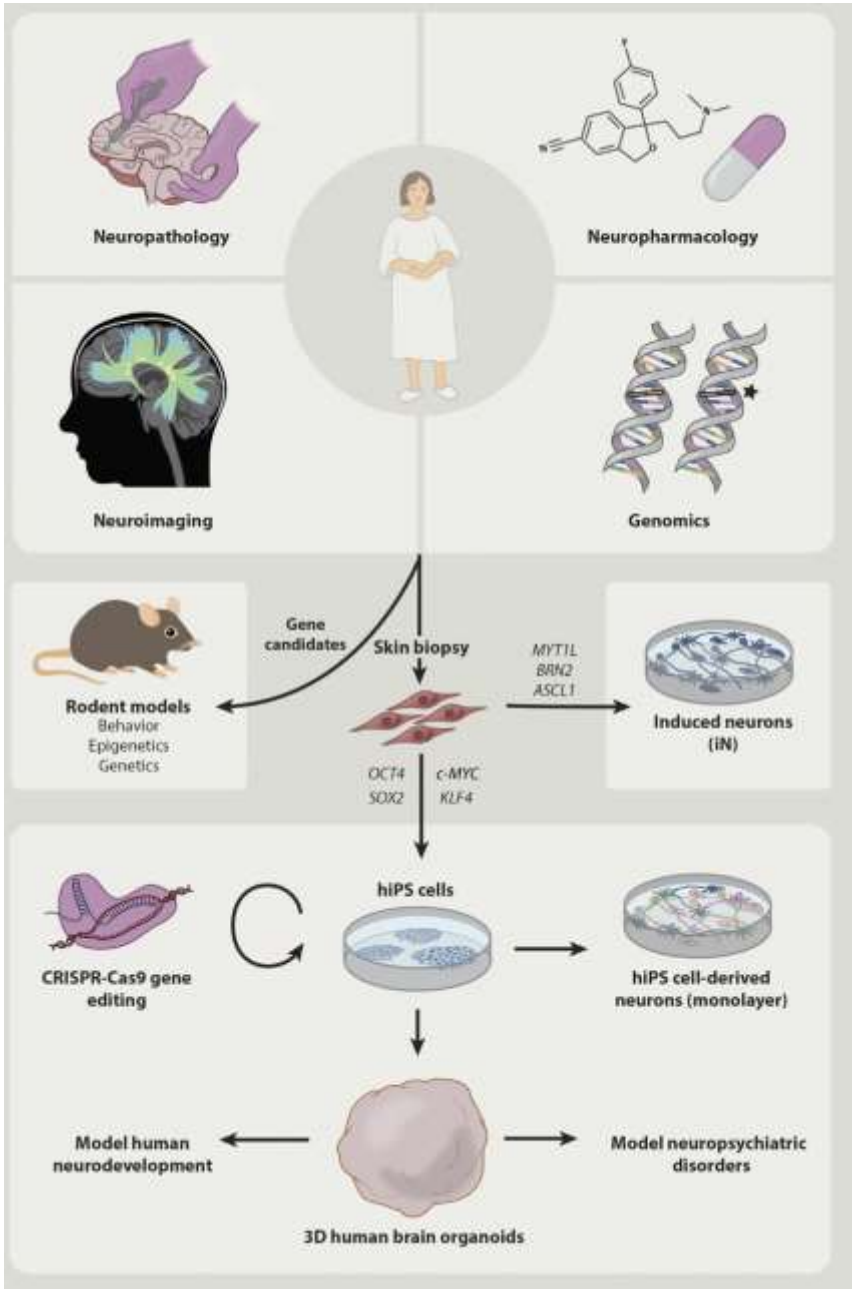


Human brain organoids can be investigated with many approaches and experimental techniques (clockwise from top) including neuronal connectivity (Deng et al., 2018; Mariani et al., 2015), xenotransplantation (Mansour et al., 2018), multi-lineage assembloids (Abud et al., 2017; Lin et al., 2018), transcriptomics (Quadrato et al., 2017) and epigenetics (Luo et al., 2016), viral transduction (Qian et al., 2016), and neurodevelopment (Bershteyn et al., 2017; Birey et al., 2017; Iefremova et al., 2017).

Amin et al; Neuron 2018

Cerebral organoids: Applications

Neurobiological mechanisms of brain disorders can be investigated with a variety of approaches in humans and model organisms. ***Somatic cells from patients can be directly reprogrammed into*** induced neurons (iNs) or ***human induced pluripotent stem (hiPS) cells.*** Using genome engineering techniques such as CRISPR-Cas9, disease-associated mutations can be introduced or corrected in hiPS cells, which can subsequently be differentiated into monolayer (2D) neuronal cultures. Alternatively, ***hiPS cells can be differentiated into brain organoids which are 3D aggregates of neural tissue that resemble human brain regions.*** Subsequently, 2D and 3D cultures can be applied to the investigation of brain disorders.



Amin et al; Neuron 2018

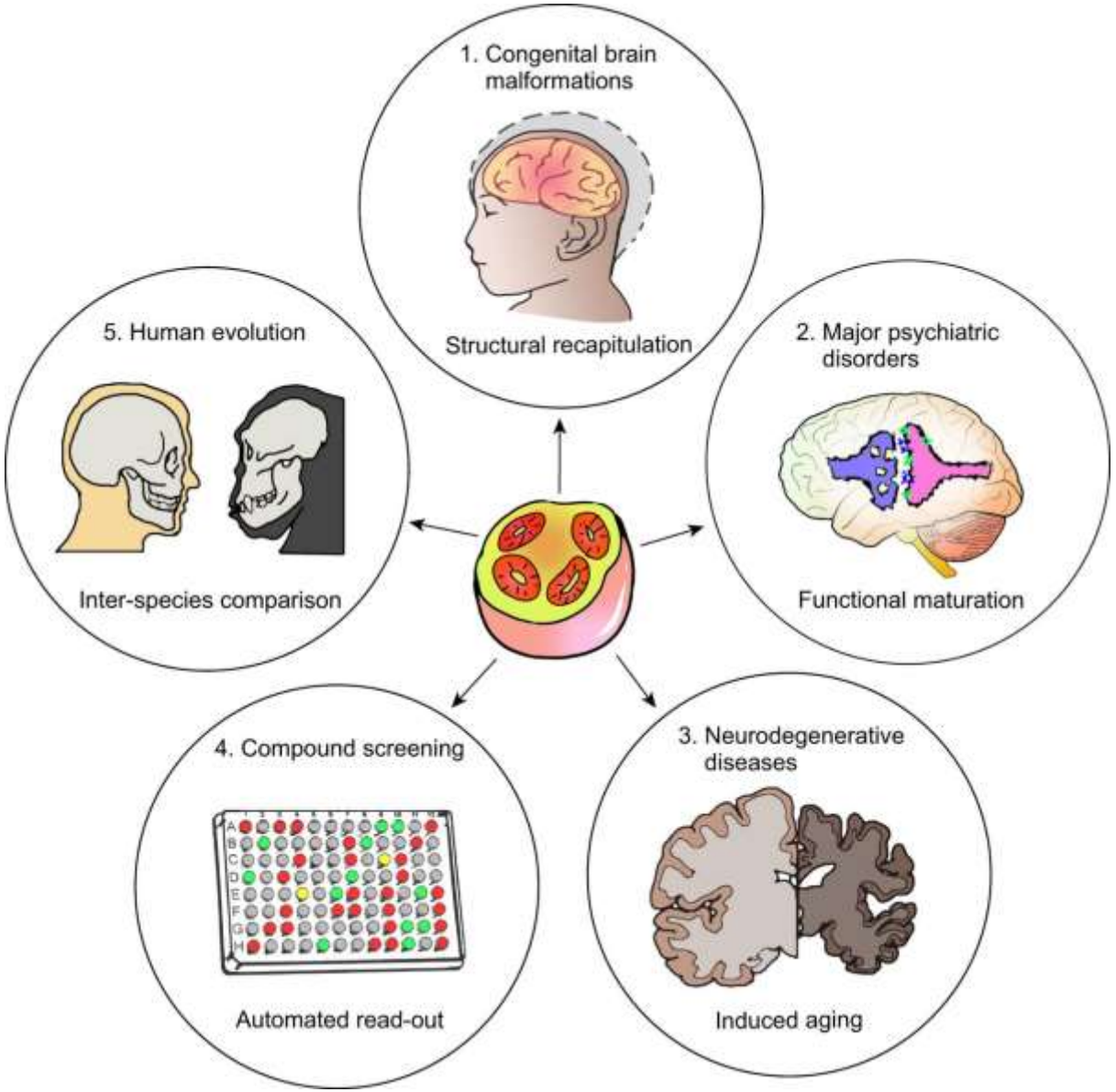
Table 1. Selected Studies Investigating Neuropsychiatric Disorders Using Human Stem Cell-Derived 3D Organoids

Disease	Cohort and Cell Lines	Organoid Type	*In Vitro* Stage	Unique Experimental Features	Phenotypes and Rescue
Miller-Dieker syndrome (Iefremova et al., 2017)	two patients (Δ17p13.3); two controls (gender/age-matched) [hiPS cell]	forebrain	4 weeks	doxycycline-inducible overexpression of *LIS1* or *YWHAE*	smaller organoids with fewer neuroepithelial loops, fewer symmetric vRG divisions, disrupted cortical niche; rescue with gene re-expression or by β-catenin activation
Miller-Dieker syndrome (Bershteyn et al., 2017)	three patients (Δ17p13.3, two lines each); three controls (four control lines total) [hiPS cell]	forebrain	5 weeks	introduction of a 5.3 Mb extrachromosomal DNA containing 17p13.3	mitotic defect in oRG, apoptosis of progenitors, cell migration defect; rescue with introduction of extrachromosomal fragment
Autosomal recessive primary microcephaly (Li et al., 2017a)	one patient (*ASPM* mutation, three lines); one control [hiPS cell]	undirected	~3 months	patient versus control organoids	smaller organoid size
Autosomal recessive primary microcephaly (Lancaster et al., 2013)	one patient (*CDK5RAP2* mutation; four lines); one control [hiPS cell]	undirected	~3 weeks	electroporation-mediated overexpression of *CDK5RAP2* and shRNA-*CDK5RAP2*	altered vRG morphology, reduced organoid size; rescue by shRNA for *CDK5RAP2*
Autism Spectrum Disorder (Mariani et al., 2015)	four patients with idiopathic ASD and macrocephaly; four familial controls (two to three lines per patient and control) [hiPS cell]	forebrain	6 weeks	lentiviral-mediated expression of shRNA-*FOXG1*	transcriptome dysregulation including *FOXG1* upregulation, increased GABAergic neuron production; rescue by shRNA knockdown of *FOXG1*
Timothy syndrome (Birey et al., 2017)	three patients (*CACNA1C* mutation, seven lines); five controls (six lines) [hiPS]	dorsal forebrain, ventral forebrain, assembloids	~2 months	forebrain assembloids; cell type specific labeling (*Dlx1/2b*::eGFP)	increased calcium following electrical depolarization, increased saltation frequency and shorter saltation length of GABAergic neurons; rescue by pharmacological modulation of L-type calcium channels
ZIKV-associated microcephaly (Qian et al., 2016)	two controls (two lines each) [hiPS cell]	forebrain	~3 months	ZIKV strains: MR766 and FSS13025 (99% amino acid similar to Brazilian ZIKV)	reduced organoid size, reduced ventricular thickness with increased ventricular lumen, non-cell autonomous apoptosis
Retinitis pigmentosa (Deng et al., 2018)	two patients (*RPGR* mutation); two controls [hiPS cell]	retinal	36 weeks	CRISPR-Cas9 genome correction of *RPGR* in hiPS cells	decreased number of rods and cones, shorter cilia, gene expression changes, increased cell death; rescue following *RPGR* gene correction
Leber congenital amaurosis (Parfitt et al., 2016)	one patient (intronic *CEP290* mutation); one control [hiPS cell]	retinal (with optic cup)	21 weeks	splice-correcting ASO for *CEP290*	defect in RPGR localization in cilia, number and length of cilia; rescue with ASO
Brain tumor (Ogawa et al., 2018)	one H9 line [hESC]	undirected	8 months	CRISPR-Cas9 recombination of HRasG12V into the *TP53* genomic locus	tumorigenesis, tumor invasiveness after xenotransplantation into the mouse CNS
Alzheimer's disease (Park et al., 2018)	ReNcell VM cells (immortalized hNPCs), hiPSC-derived hNPCs	neural cell line in microfluidic chamber	9 weeks	viral transduction of hNPCs with an APP variant carrying multiple FAD mutations; co-culture with the human immortalized microglia SV40 cell line	increased Aβ aggregation and greater microglia recruitment in FAD cultures; reduction in microglia migration speed with anti-CCL2 neutralizing antibodies; microglia induce neuronal toxicity in FAD cultures

Relevant experimental features are highlighted, including the type and number of independent human cell lines, the specific type of brain organoid differentiation, the duration of organoid culture for disease phenotyping, and key disease-related phenotypes identified, including any phenotypic rescues. hiPS cell, human induced pluripotent stem cell; hESC, human embryonic stem cell; hNPC, human neural progenitor cell; vRG, ventral radial glia; oRG, outer radial glia; ASO, anti-sense oligonucleotide; APP, amyloid precursor protein; FAD, familial Alzheimer's disease.

Amin et al; Neuron 2018

Cerebral organoids: Applications



(1) Brain organoids have proven to be particularly informative for modeling ***congenital brain malformations*** caused by genetic deficits or infectious disease, because the organoid cytoarchitecture provide a direct read-out for disease-relevant phenotypes. ***(2)*** Primitive microcircuits are detected in brain organoids (Birey et al., 2017), but further promoting functional maturation will be key for ***modeling psychiatric disorders such as autism and schizophrenia.*** **(3)** It remains challenging ***to model age-dependent neurodegenerative diseases*** with current brain organoids, because they mimic mostly embryonic brain development. A method of ***artificially inducing aging in vitro could potentially allow organoids to represent disease-relevant phenotypes.*** **(4)** The ability to generate brain organoids in large quantities with high consistency raises the possibility of ***using organoids for compound screening and subsequent validation.*** The development of automated high-throughput platforms could expedite such advances. ***(5)*** Organoids offer the unique opportunity to understand the basis of human brain formation and evolution in comparison to other species. For instance, ***brain samples from great apes are largely inaccessible, but organoids generated from great ape iPSCs can be compared with human cell-derived organoids to discover uniquely human features.***

Qian et al; Development 2019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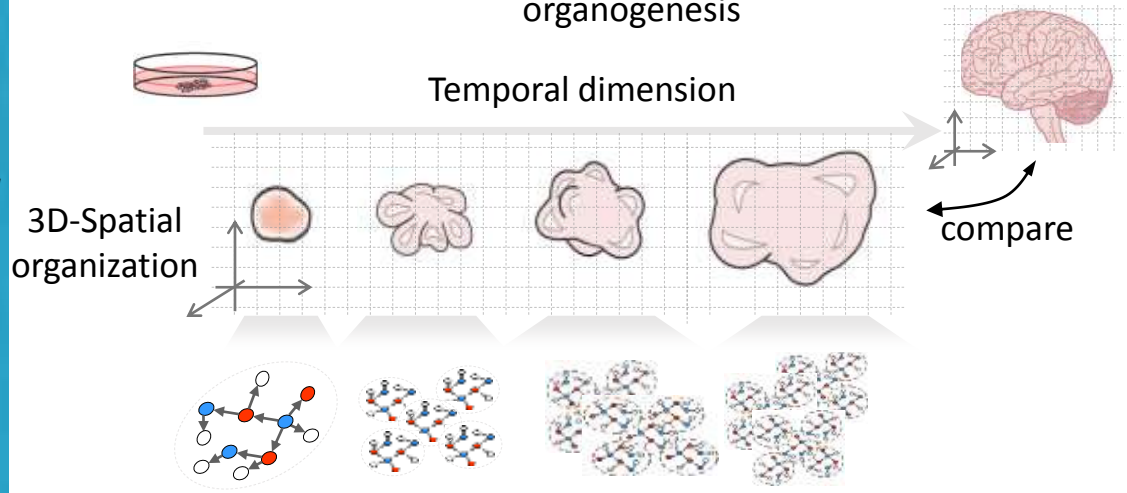
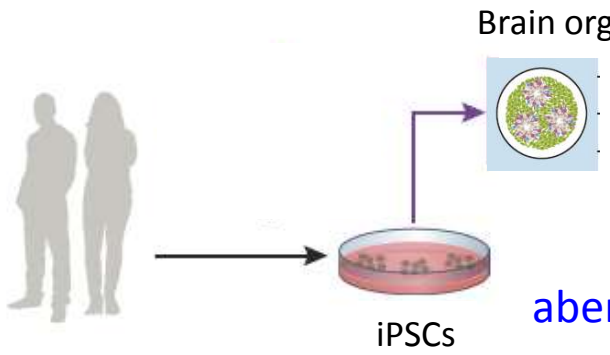
Systems Biology of reconstituted "mini brains"

4-dimensional cell fate decisions implicated in brain organogenesis



Spatial gene regulatory networks (GRNs)

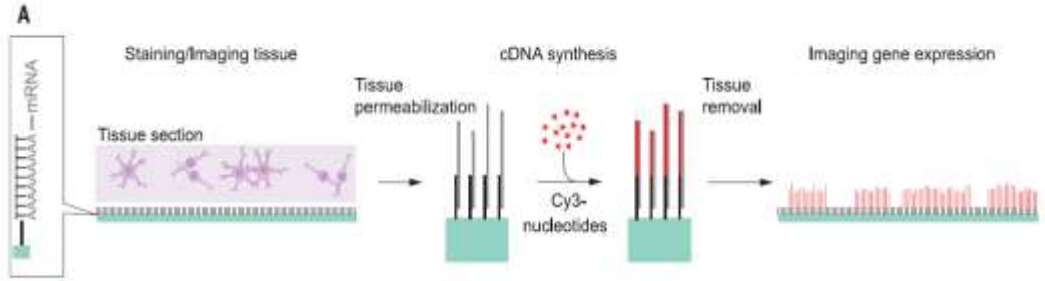
Systems Biology of reconstituted "mini brains" from human iPSCs samples issued from Alzheimer's Disease patients



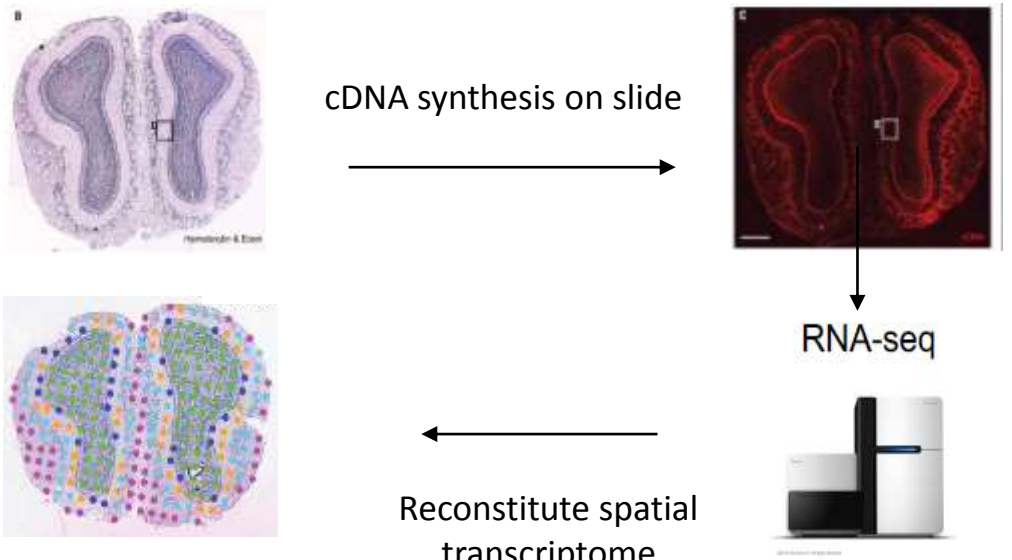
aberrant GRNs?

Spatial transcriptomics on tissue sections

(Stahl et al; Science 2016)



Glass slides containing barcoded-polyT oligos: spatial information



Ethical considerations

Human tissues in a dish: The research and ethical implications of organoid technology

Annelien L. Bredenoord, Hans Clevers, Juergen A. Knoblich*

Science 2017

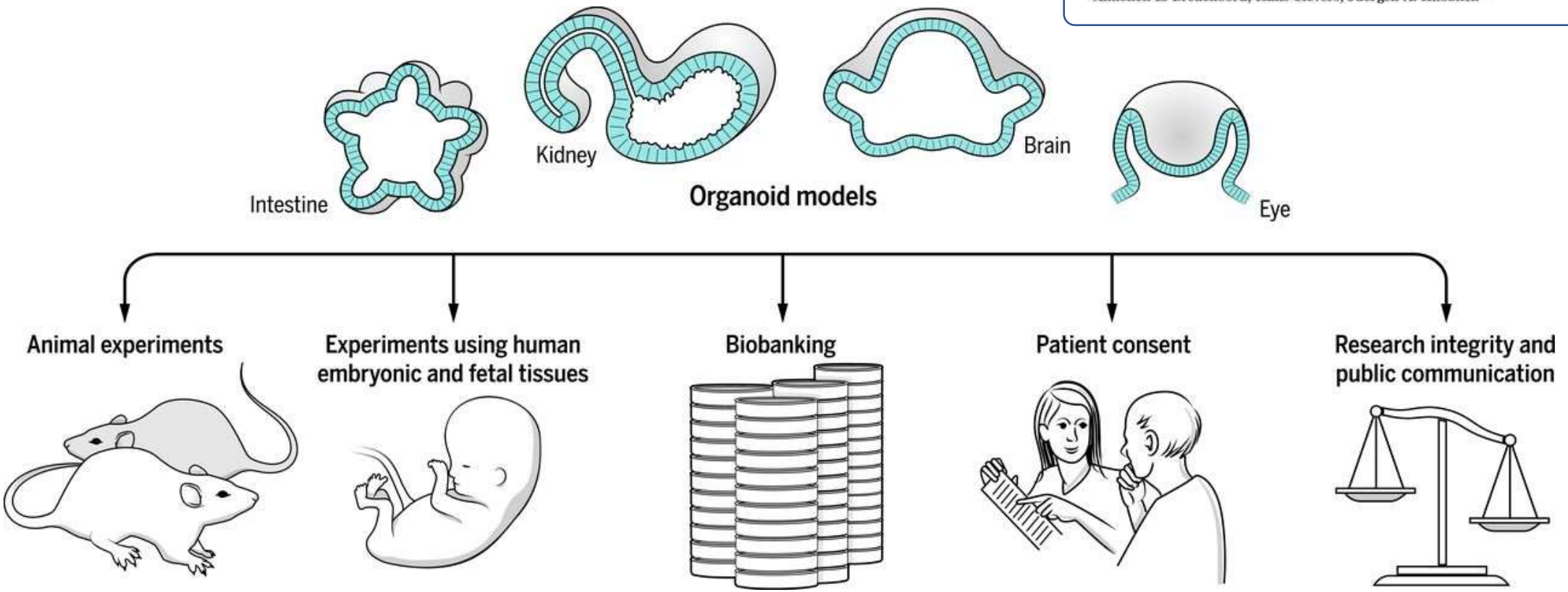


Table 1. Scientific and ethical comparison of animal models, human embryo tissues, and organoid models.

	Biological considerations	Ethical considerations
Animal models	Modeling of complex organ interactions	Animal research is ethically controversial
	Models include immune system, blood vessels	Reduction, refinement, and/or replacement of animal experiments is a commonly accepted goal
	Results often not transferrable to humans	
Human embryos and fetuses	Experimental limitations include reduced numbers because of low availability	Diversity of views regarding the moral acceptability of using and creating embryos for research
	Necessary for verification of organoid results and human reagents (antibodies)	Most countries allow research on embryonic and fetal tissues under strict conditions
	Organoid research might increase rather than decrease the need	
Organoid models	Close to unlimited availability	No animal experiment, no direct use of human embryos and fetuses
	Reprogramming and genome editing techniques allow unprecedented personalization of experiments	Current culture protocols include animal-derived reagents (Matrigel)
	Limited by variability and lack of predefined axis	Some protocols require the use of human embryonic stem cells
	No blood vessels, no immune system	Organoids might require specific patient consent
	Biobanking necessary for some types of organoids	Biobanking raises specific ethical issues
		Frontier science: specific responsibilities for scientists in the field

Human tissues in a dish: The research and ethical implications of organoid technology Science 2017

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As Brain Organoids Mature, Ethical Questions Arise

Inserting human "mini-brains" into rodents has the potential to broaden scientists' understanding of neurological disease, but raises quandaries about consciousness.

TheScientist
EXPLORING LIFE, INSPIRING INNOVATION


Marco A. Mendoza


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Bruno Colombo

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