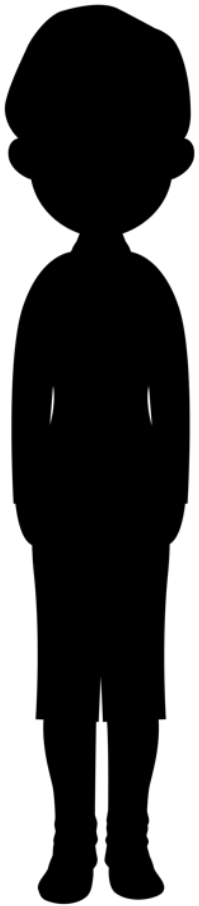
Next generation of neuronal cultures: modeling the brain with self assembled organoids and 3d bioprinted constructs

preclinical phase

clinical phase

90%

Failure rate*



*Tuft University (2016)

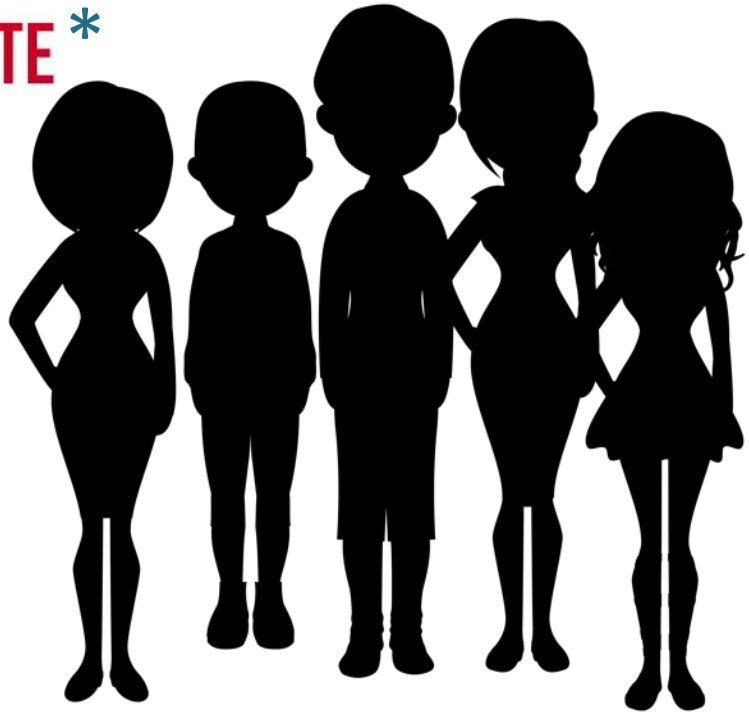
preclinical

Phase i/ii

Phase III

90%
FAILURE RATE

50%
FAILURE RATE*



*Parexel (2016)

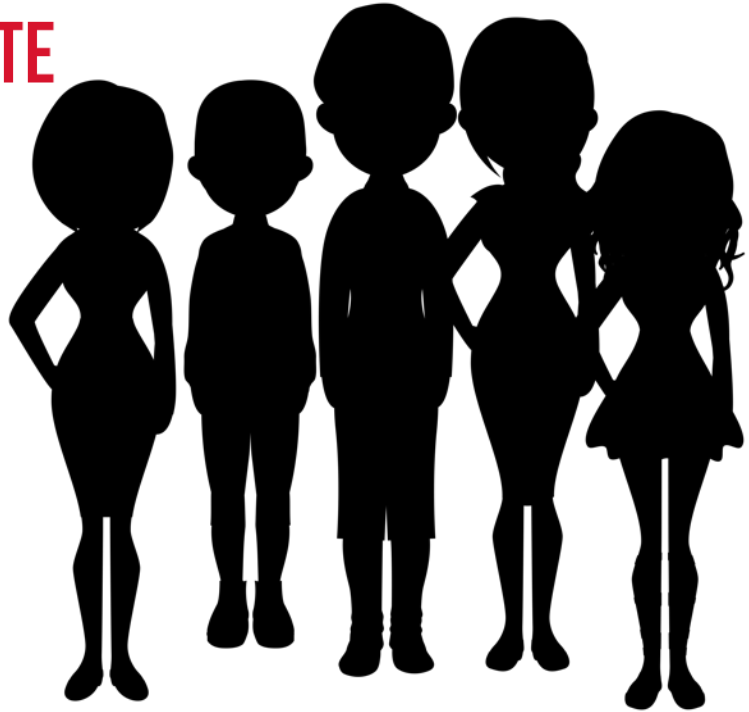
preclinical

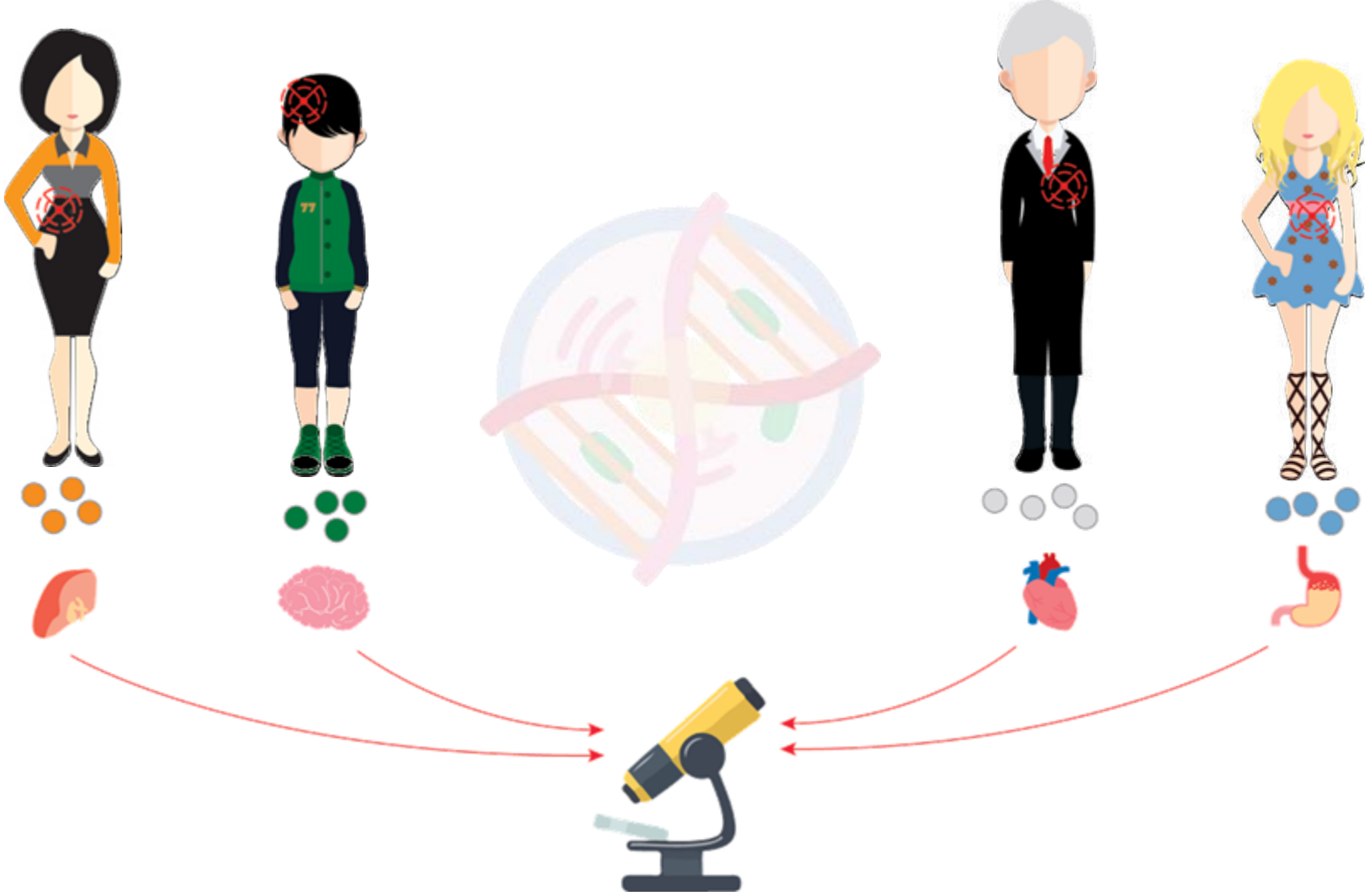
Phase i/ii

Phase III

90%
FAILURE RATE

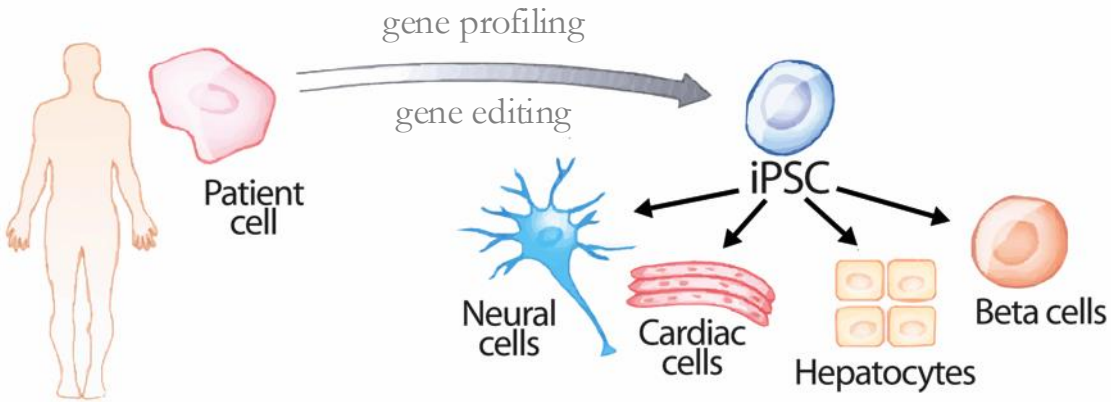
50%
FAILURE RATE



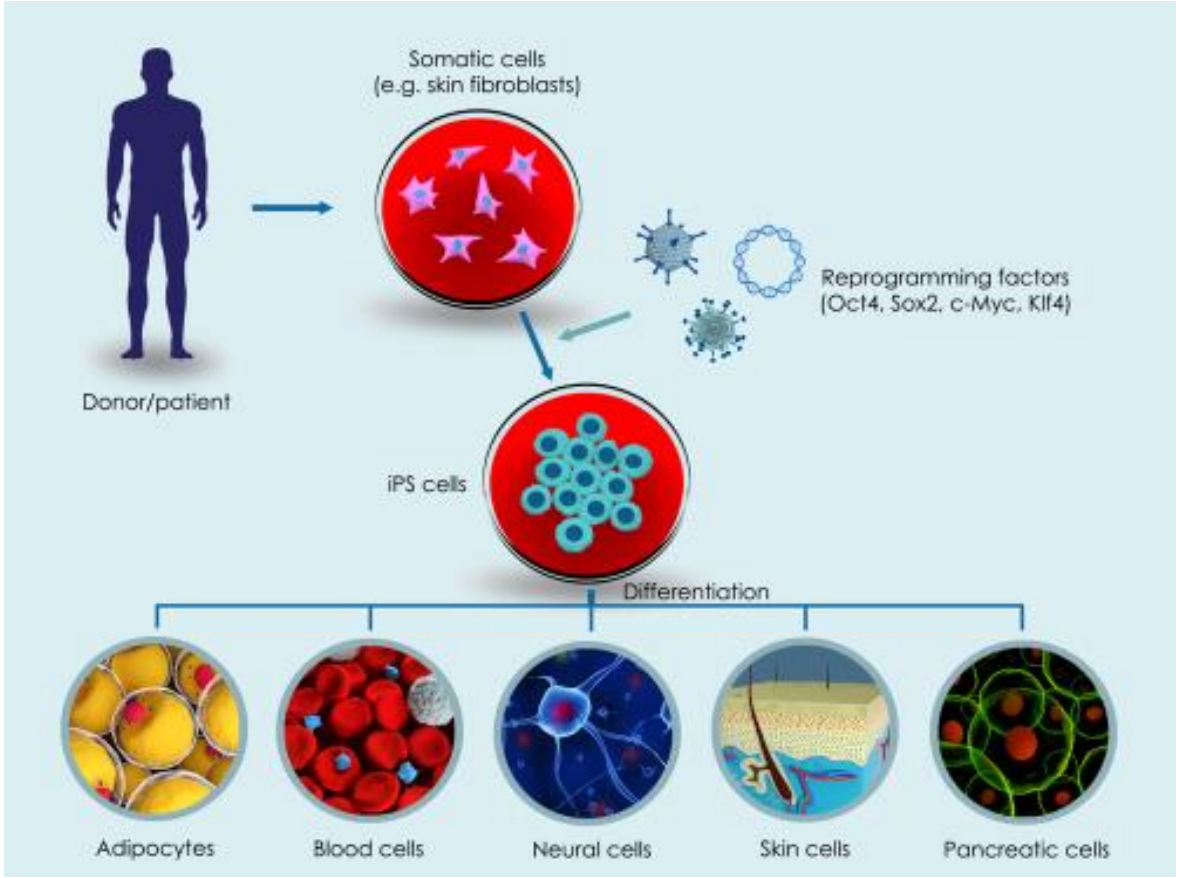


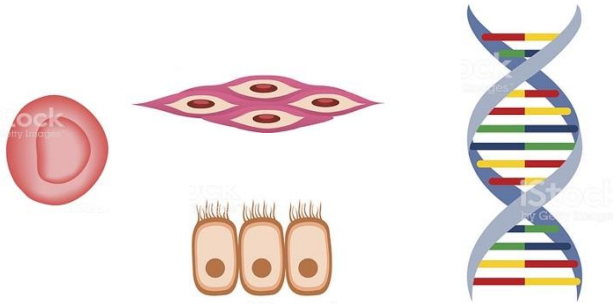
New tools- bricks

Induced Pluripotent Stem Cells (iPSCs)



Transforming adult cells into stem cells





Shifting paradigms

2012 - Nobel Prize in Physiology or Medicine

Sir John B. Gurdon and Shinya Yamanaka

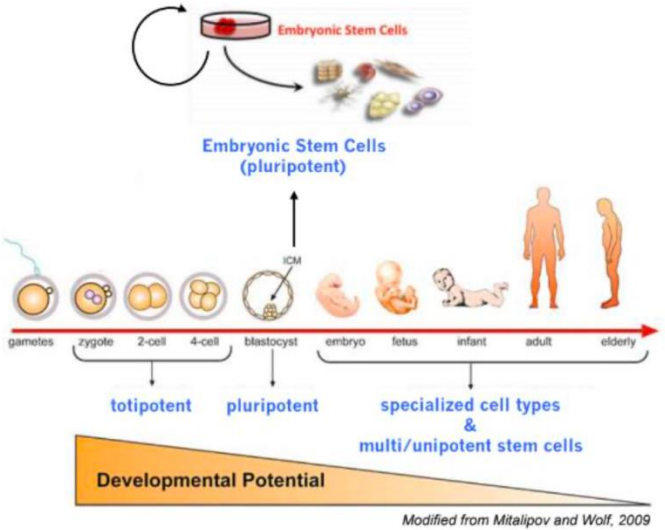
"for the discovery that mature cells can be reprogrammed to become pluripotent."

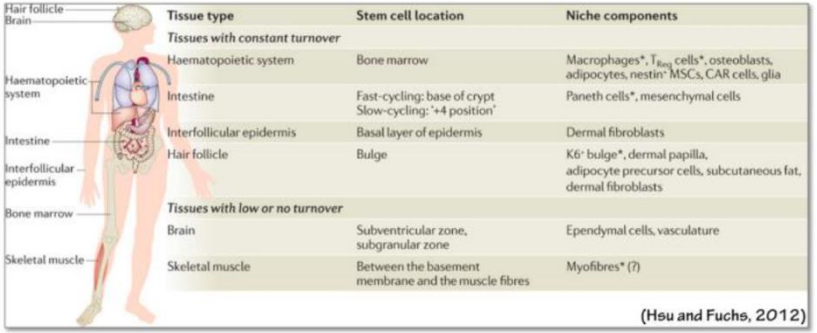
2020 - Nobel Prize in Chemistry

Emmanuelle Charpentier and Jennifer A. Doudna

"for the development of a method for genome editing."

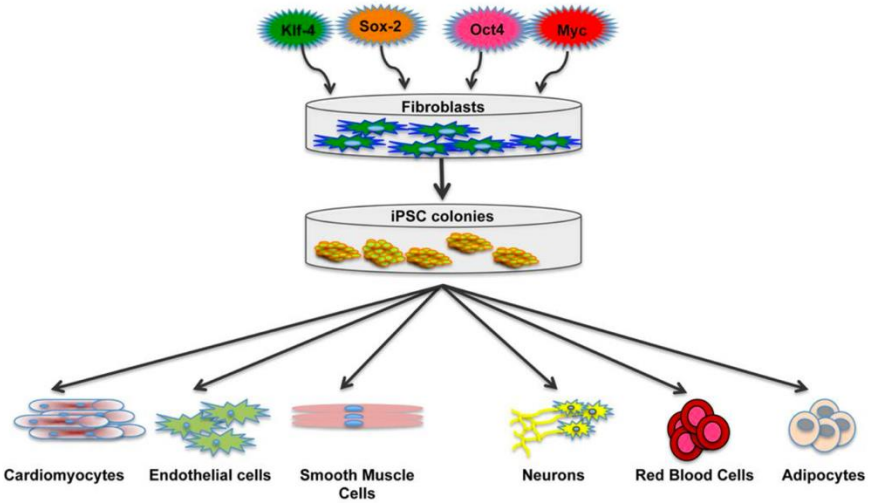
Induced Pluripotent Stem Cells (iPSCs)





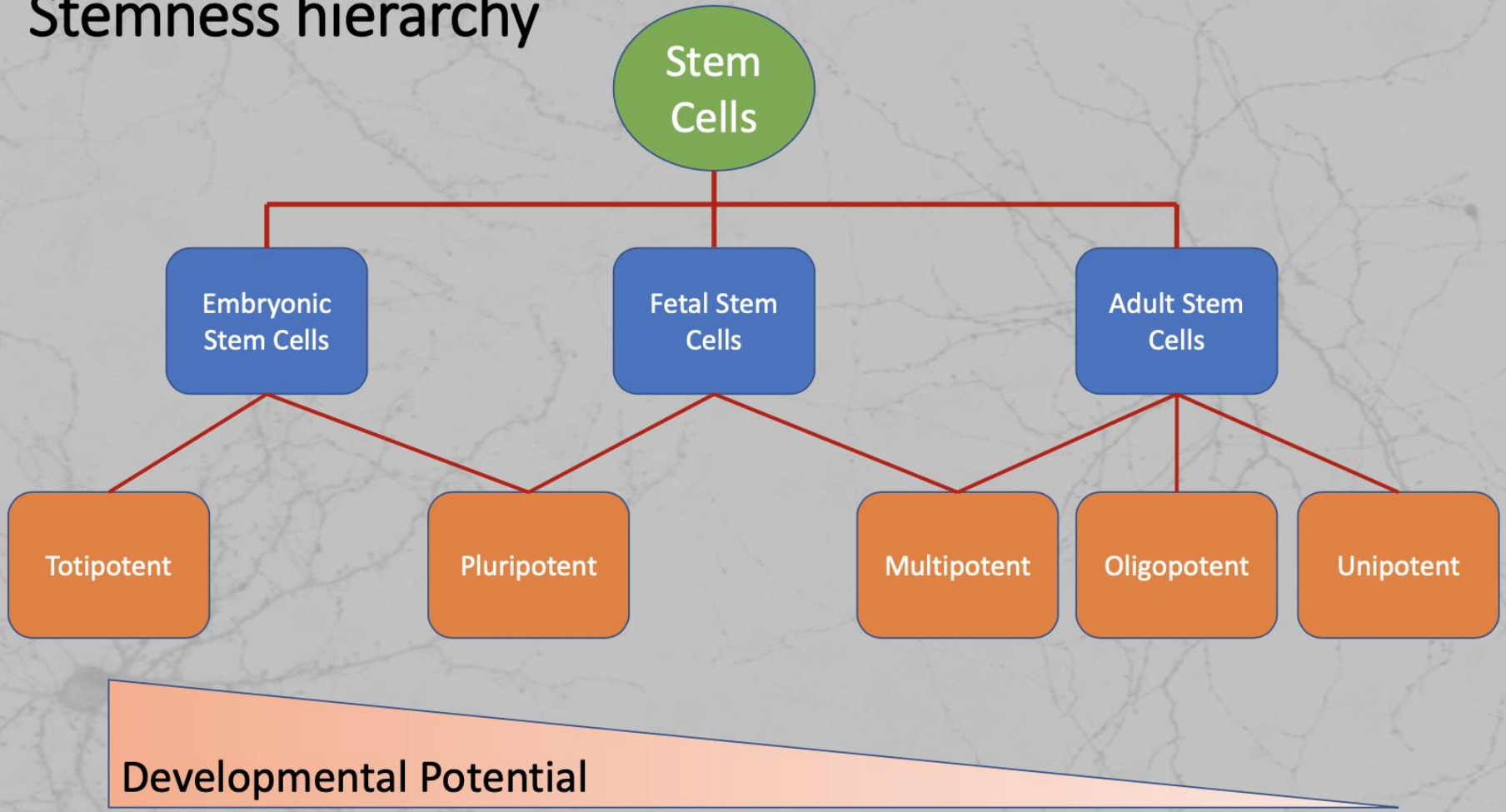
Tissue type	Stem cell location	Niche components
Tissues with constant turnover		
Haematopoietic system	Bone marrow	Macrophages*, T_{Reg} cells*, osteoblasts, adipocytes, nestin$^+$ MSCs, CAR cells, glia
Intestine	Fast-cycling: base of crypt Slow-cycling: '+4 position'	Paneth cells*, mesenchymal cells
Interfollicular epidermis	Basal layer of epidermis	Dermal fibroblasts
Hair follicle	Bulge	K6$^+$ bulge*, dermal papilla, adipocyte precursor cells, subcutaneous fat, dermal fibroblasts
Tissues with low or no turnover		
Brain	Subventricular zone, subgranular zone	Ependymal cells, vasculature
Skeletal muscle	Between the basement membrane and the muscle fibres	Myofibres* (?)

(Hsu and Fuchs, 2012)

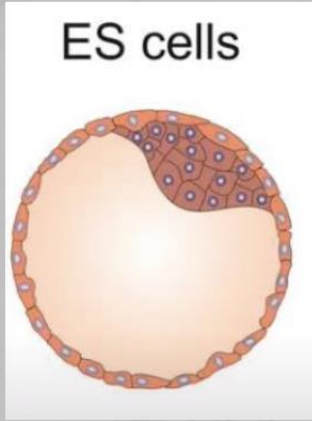


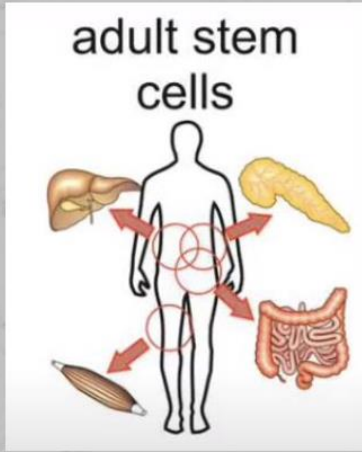
The human induced pluripotent stem cells represent an easy accessable, convenient and valuable alternative to embryonic stem cells and other in situ stem cell populations.

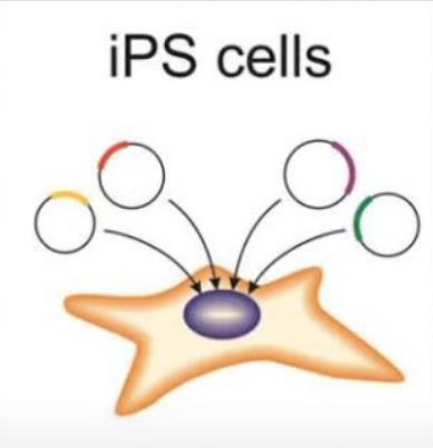
Stemness hierarchy



Stem cell types







Pros:
- Highly expandable
- Pluripotent

Cons:
- Tumorrisk
- Geneticinstability
- Ethicalissue

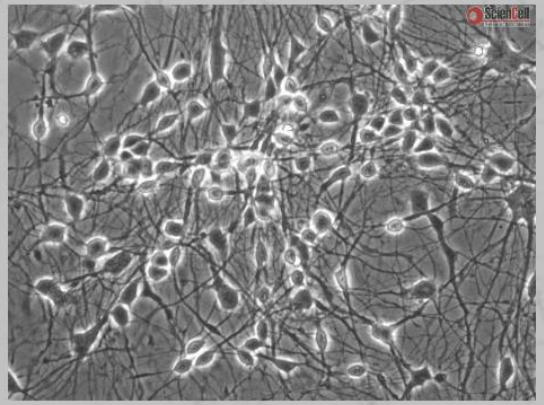
Pros:
- Multipotent
- Low tumor risk
- Tissue specification

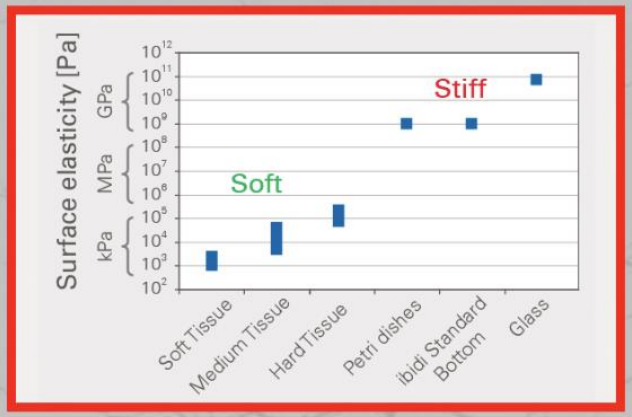
Cons:
- Invasiveness
- Inefficient in vitro expansion

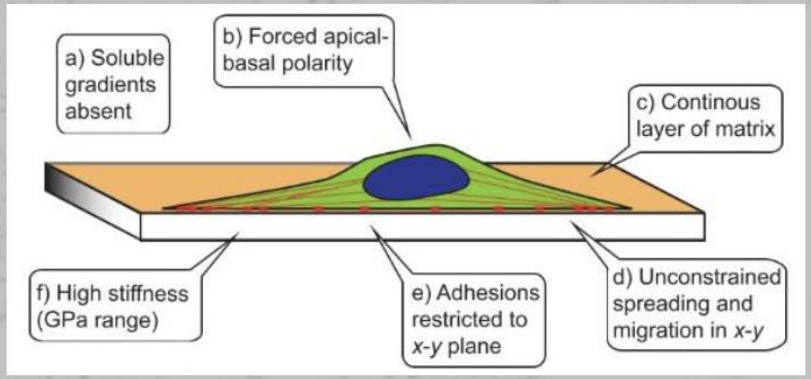
Pros:
- Highly expandable Pluripotent
- Reprogrammed

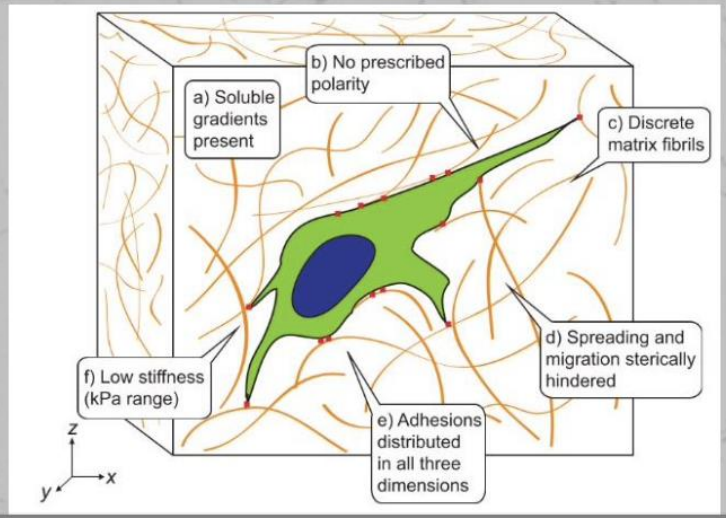
Cons:
- Tumor risk
- Genetic instability
- Reprogramming approach

Culturing system shift

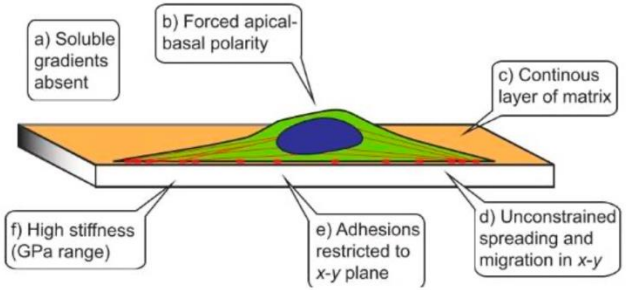


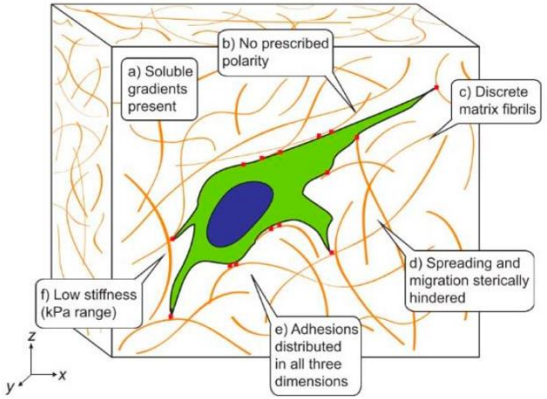


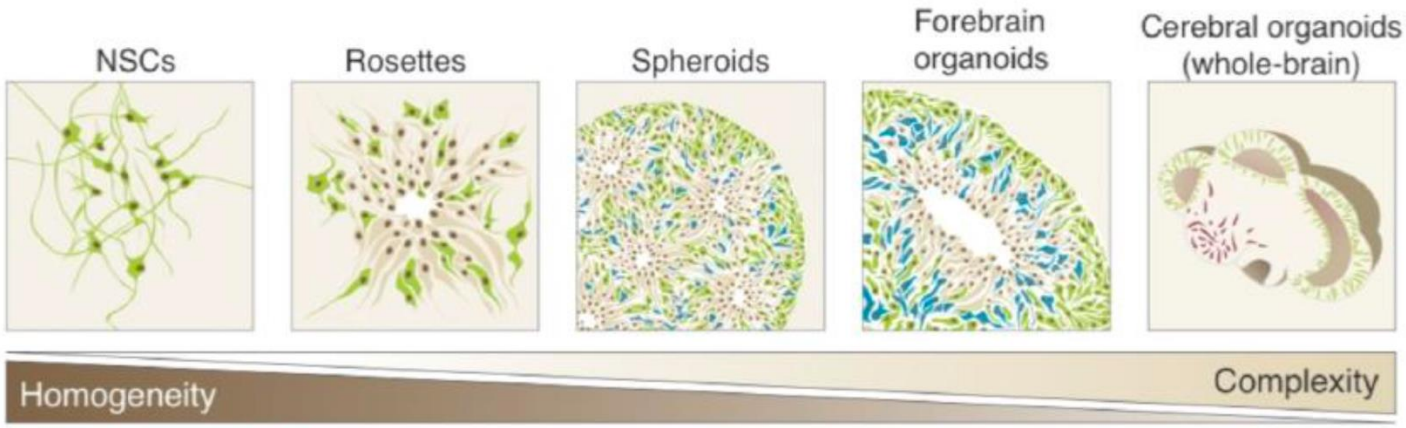




From 2d to 3d cell cultures





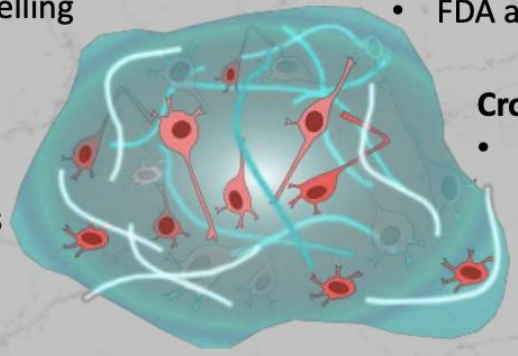


The importance of the 3D matrix

Mechanical properties
- Tunable to reach the elasticity of the desired tissue
- Mesh size, porosity, crosslinking density, swelling

Biocompatibility
- No or negligible toxic effects
- Sterilization
- FDA approval

Mass transport
Continuous exchange of nutrients, proteins, gases and waste products

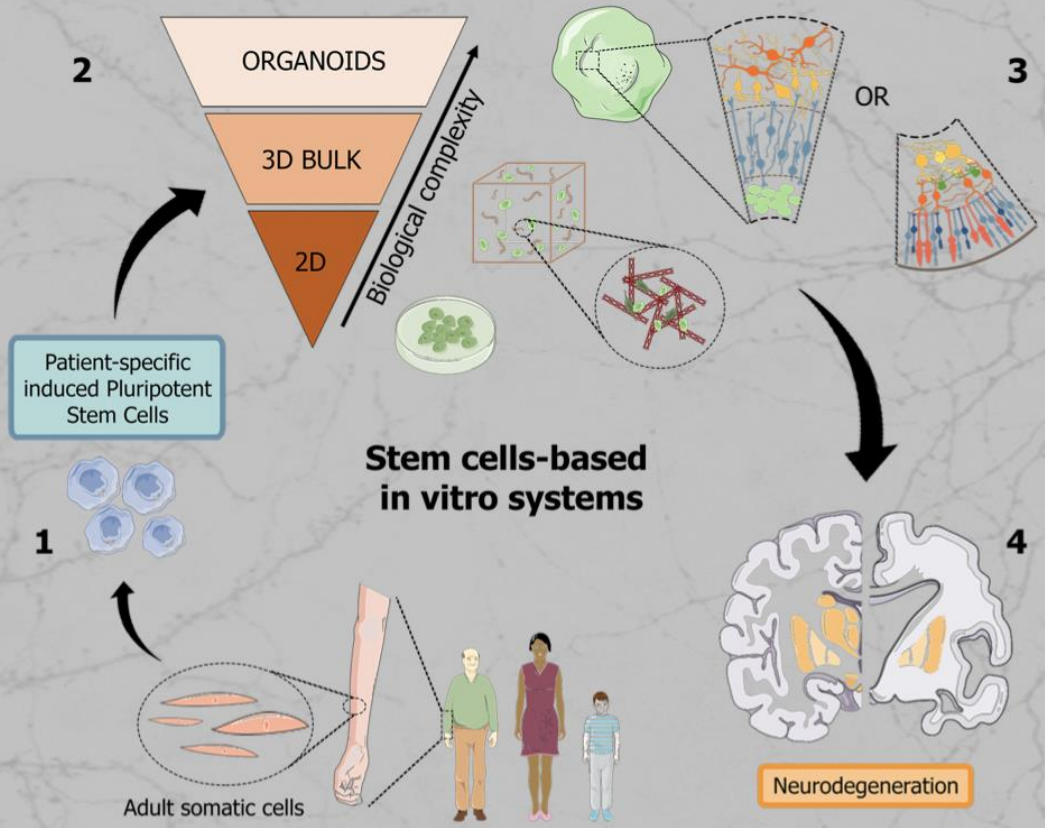


Crosslinking in presence of cells
- Limited noxious effects on cells

Mimicking microenvironment
- Mimicking the native extracellular matrix (ECM)
- Allowing the cells to produce their own ECM

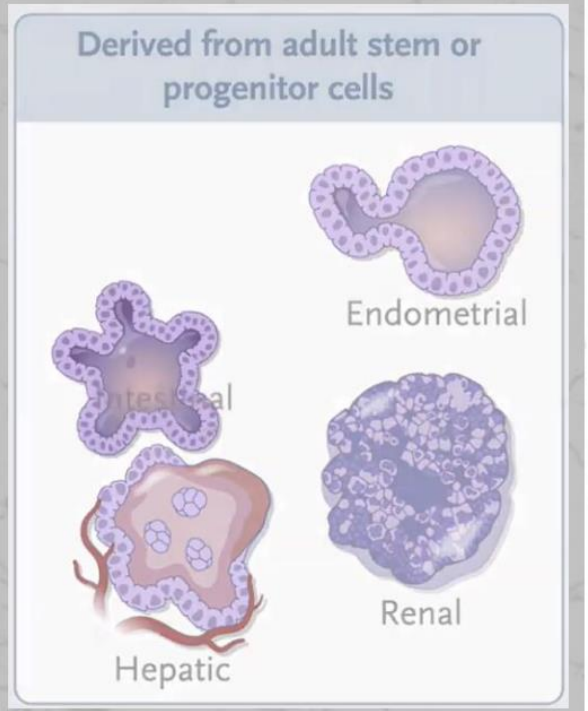
Degradability
- Control of degradation kinetics/stability

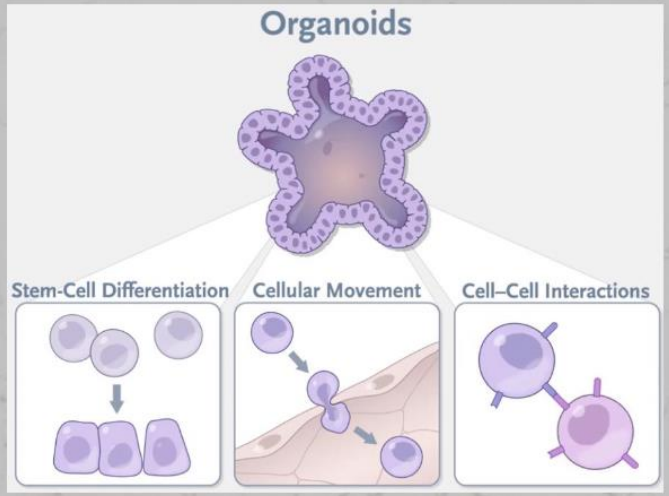
Organoid technology



Doi: 10.3389/fnins.2020.00655

Organoid technology





Organoid features:

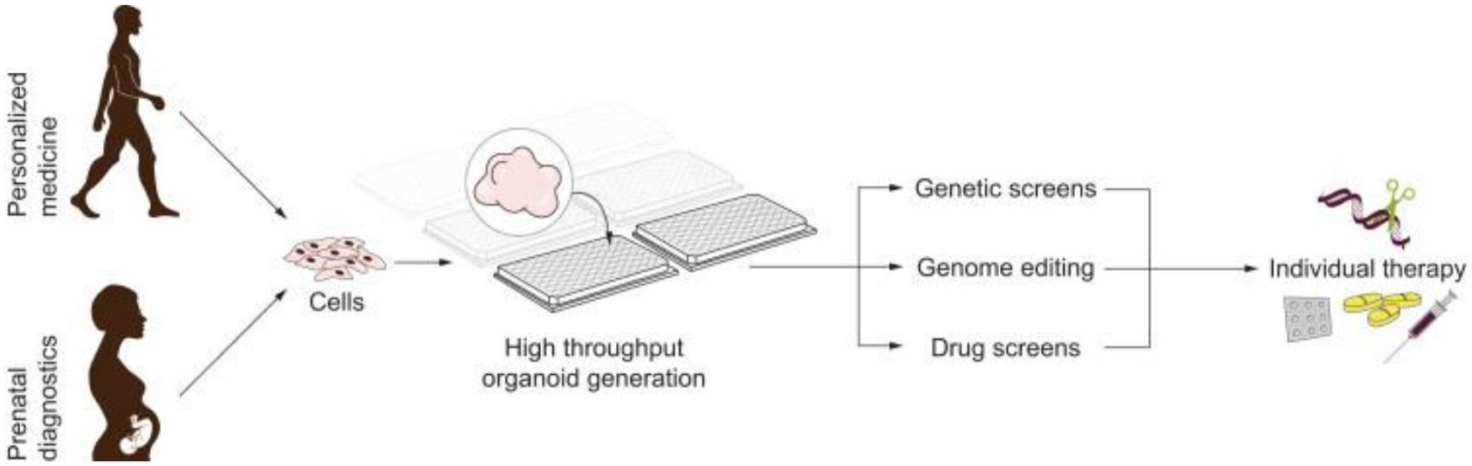
- untransformed
- 3 dimensional with realistic micro-anatomy
- highly proliferative and expandable
- recapitulate functions of their parent tissue
- very high genetic stability

https://www.youtube.com/watch?v=2SG5ivm6jkw

Modeling human brain development using iPSCs

- The human brain is one of the most complex organs in animal kindom, both structurally and functionally.

- hiPSCs can be used to have access to a physiologically relevant human model for drug discovery, cell therapy validation and neurological disease research.



What are cerebral organoids?

- A cerebral organoid describes artificially grown, *in vitro*, miniature organs resembling the brain.
- They are created by culturing human pluripotent stem cells in a three-dimensional rotational bioreactor and develop over a course of months .

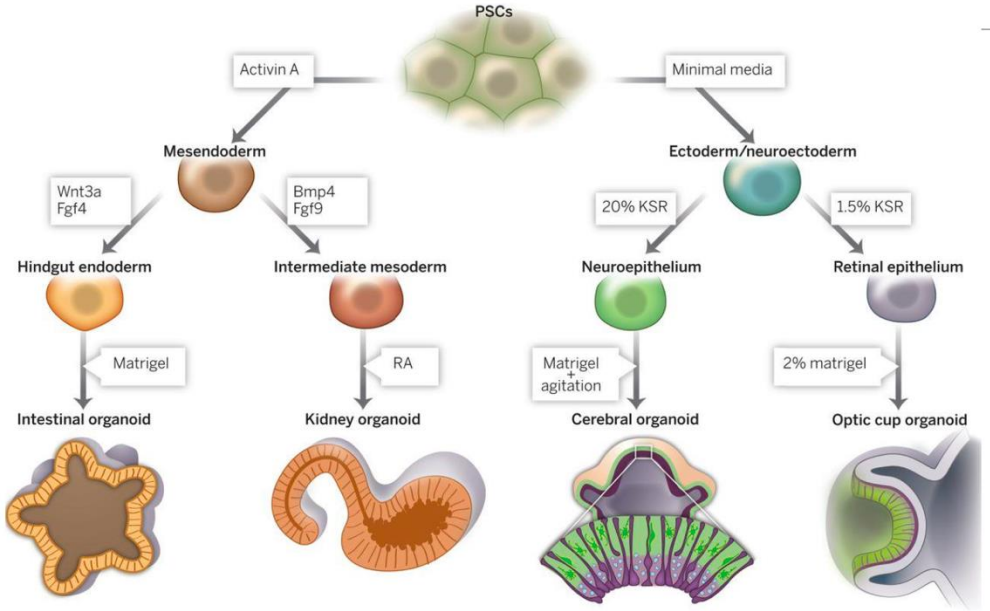
Review article

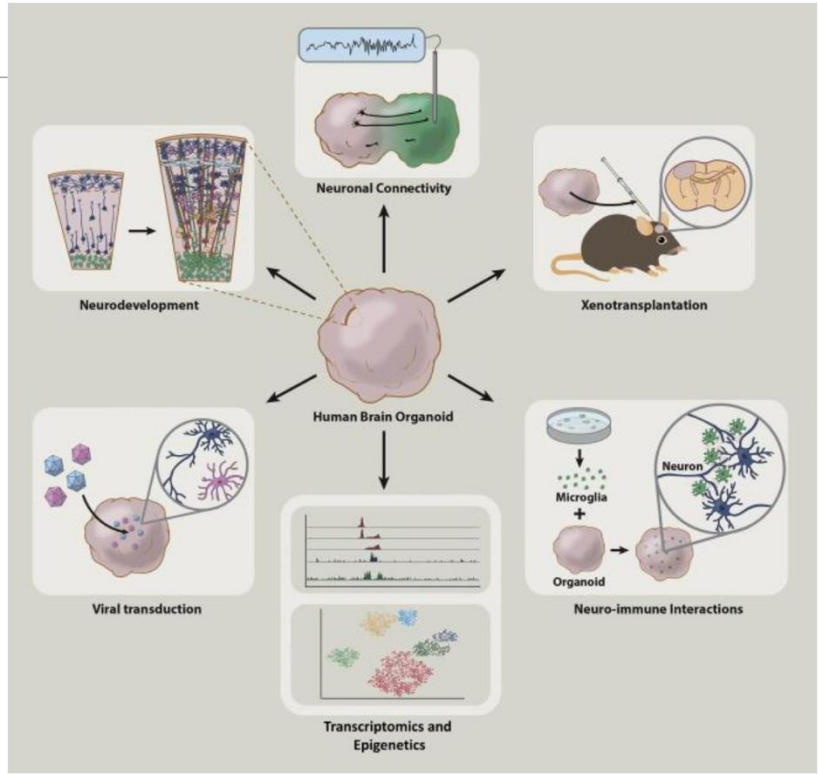
Dishing out mini-brains: Current progress and future prospects in brain organoid research

Iva Kelava, Madeline A. Lancaster*

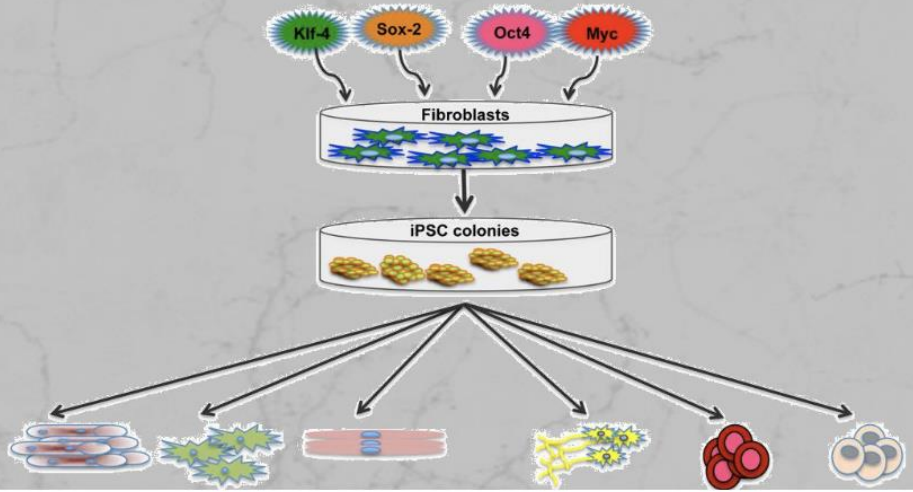
MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus, Francis Crick Avenue, CB2 0QH Cambridge, United Kingdom

What are cerebral organoids?



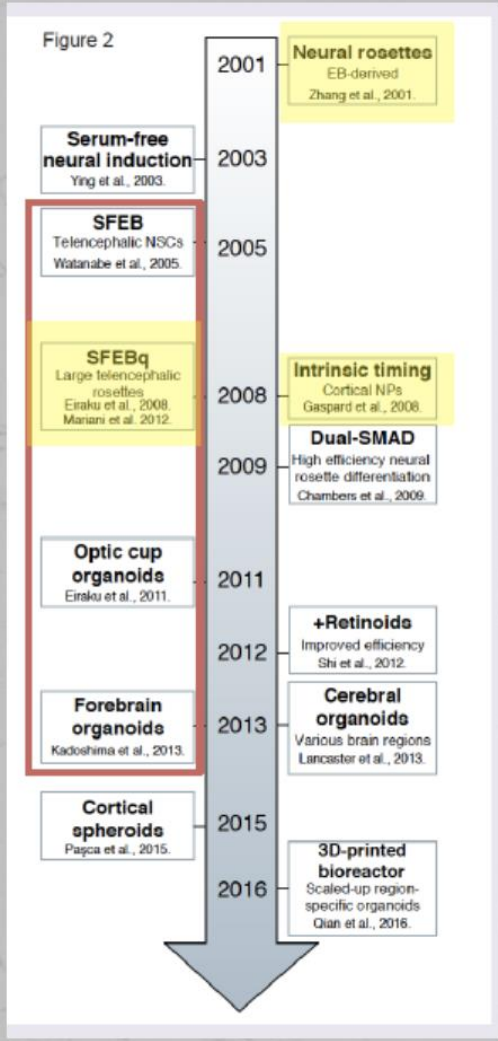


Cerebral organoid

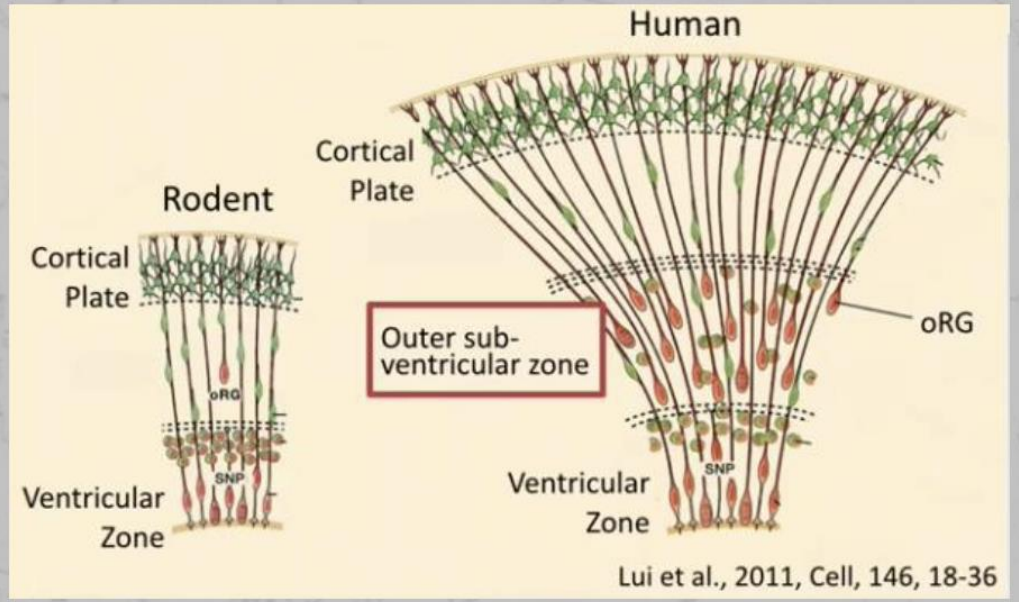


Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

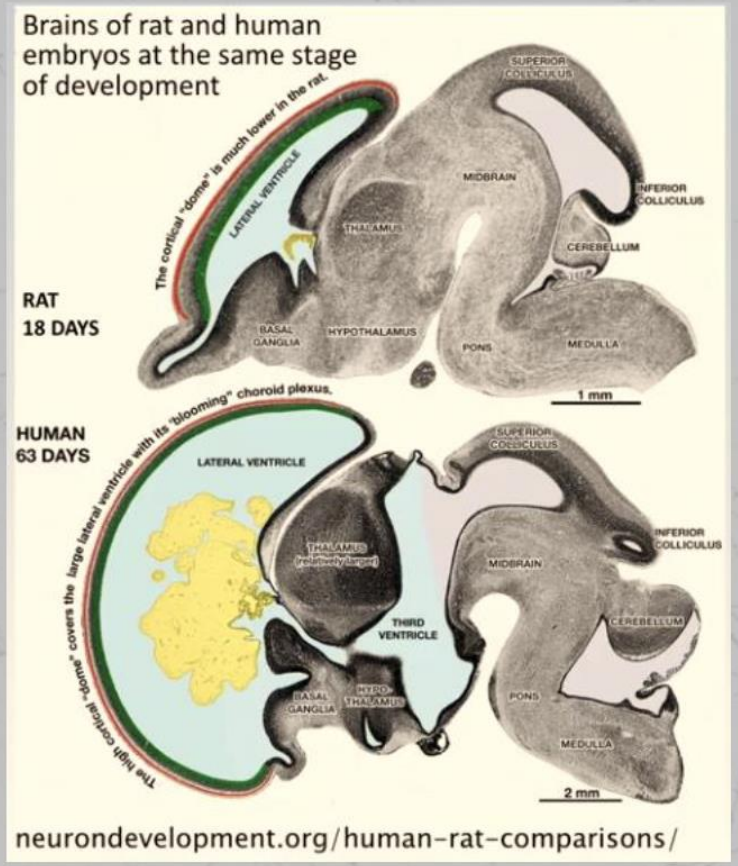
Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1]Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2]CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024



Cortical plate development

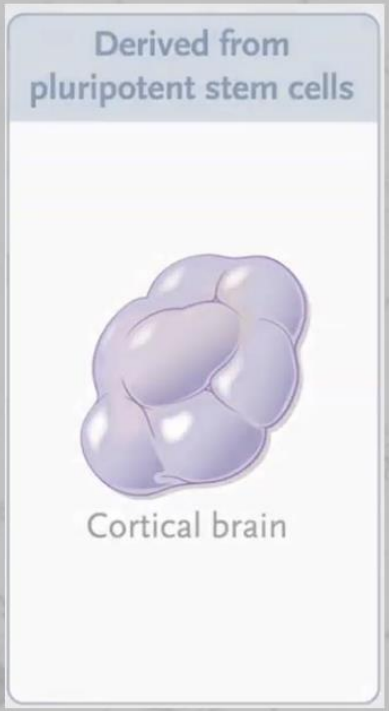


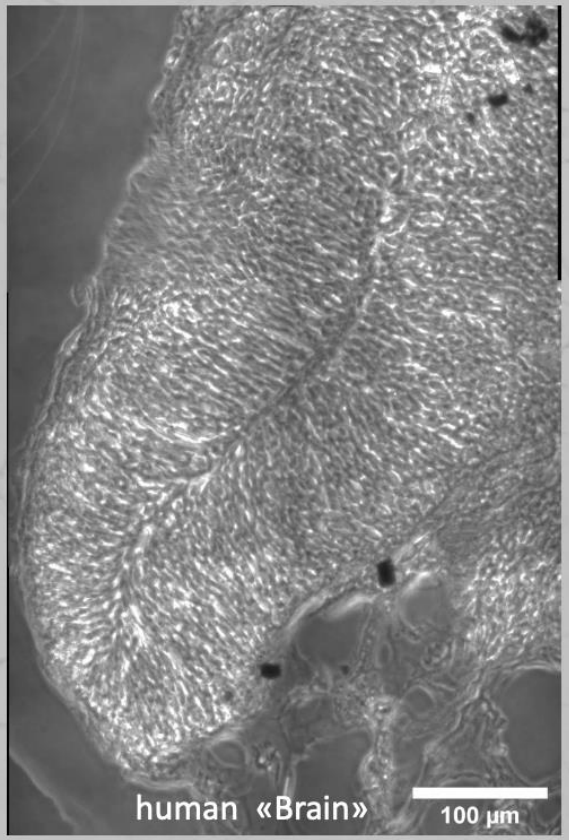
Lui et al., 2011, Cell, 146, 18-36



neurondevelopment.org/human-rat-comparisons/

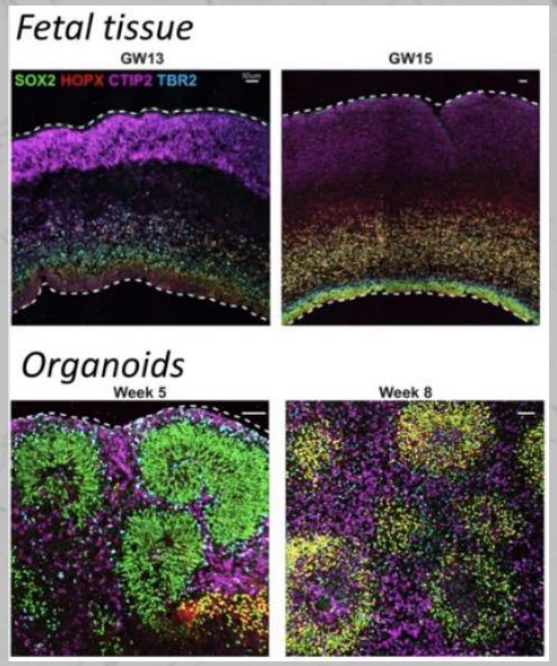
Cerebral organoid



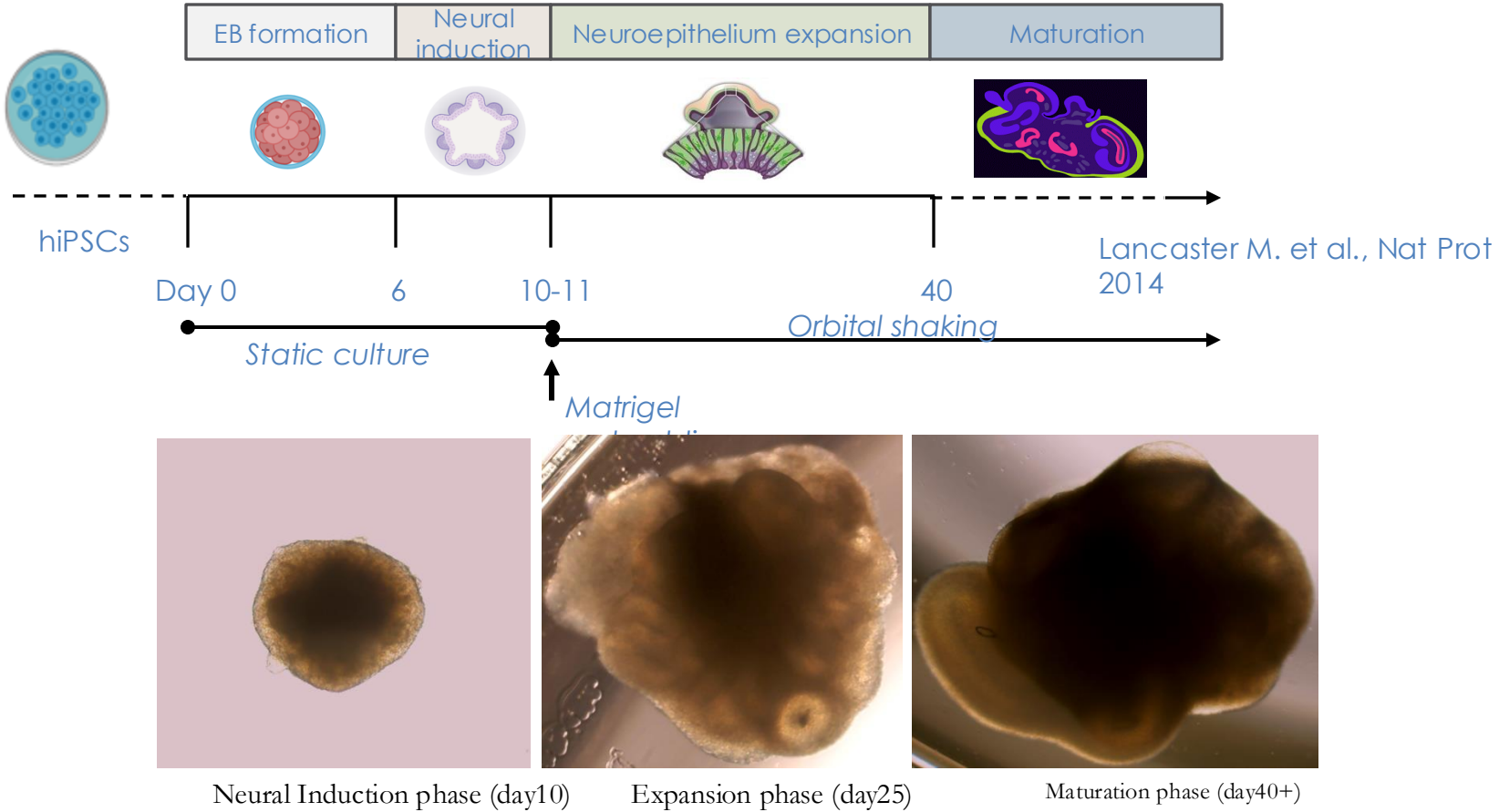


Advantages:

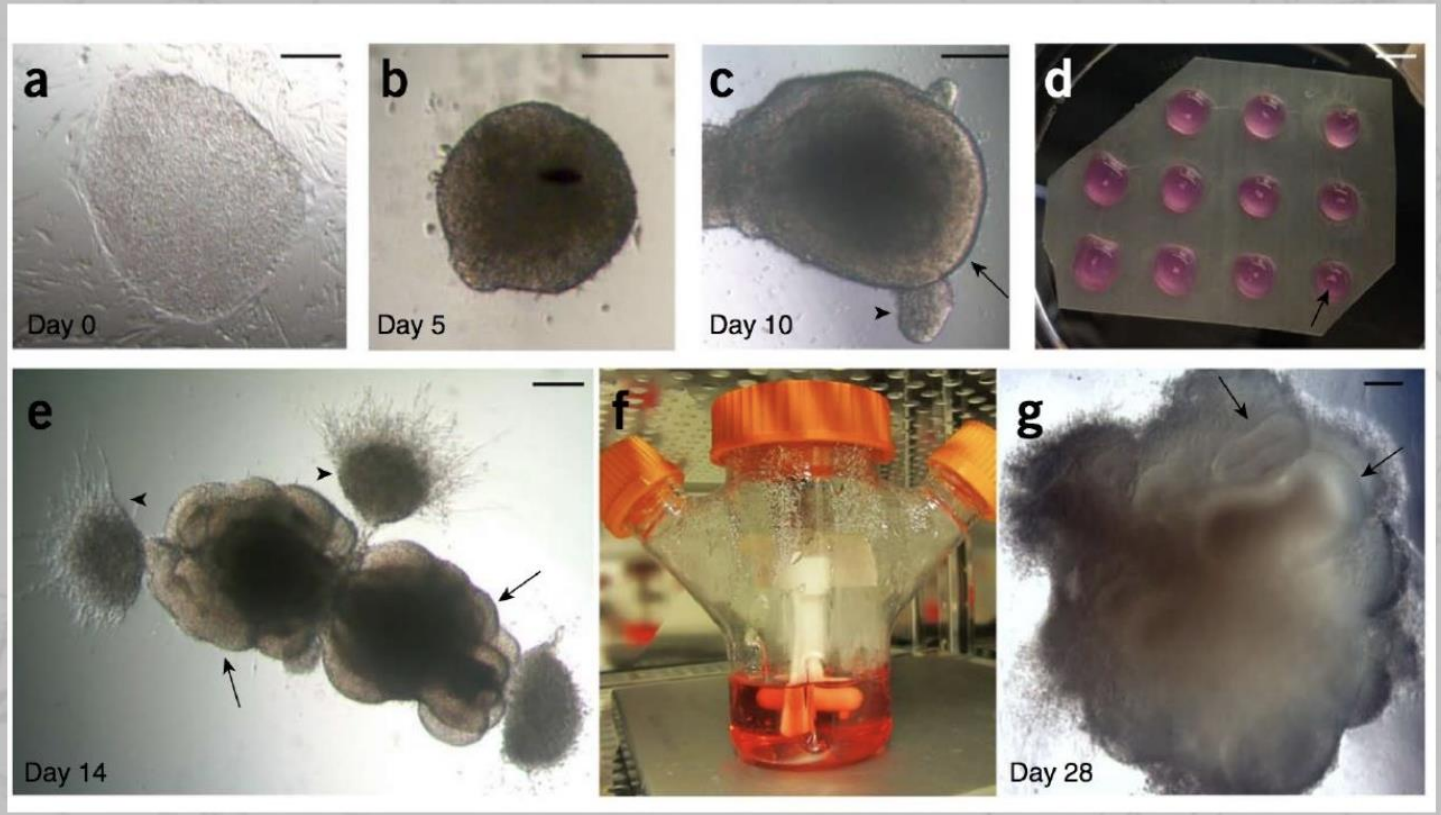
- Reduced experimental complexity and costs
- Suitable for live imaging exps
- More accurate model of human brain development and disease



Generation of human cortical organoids

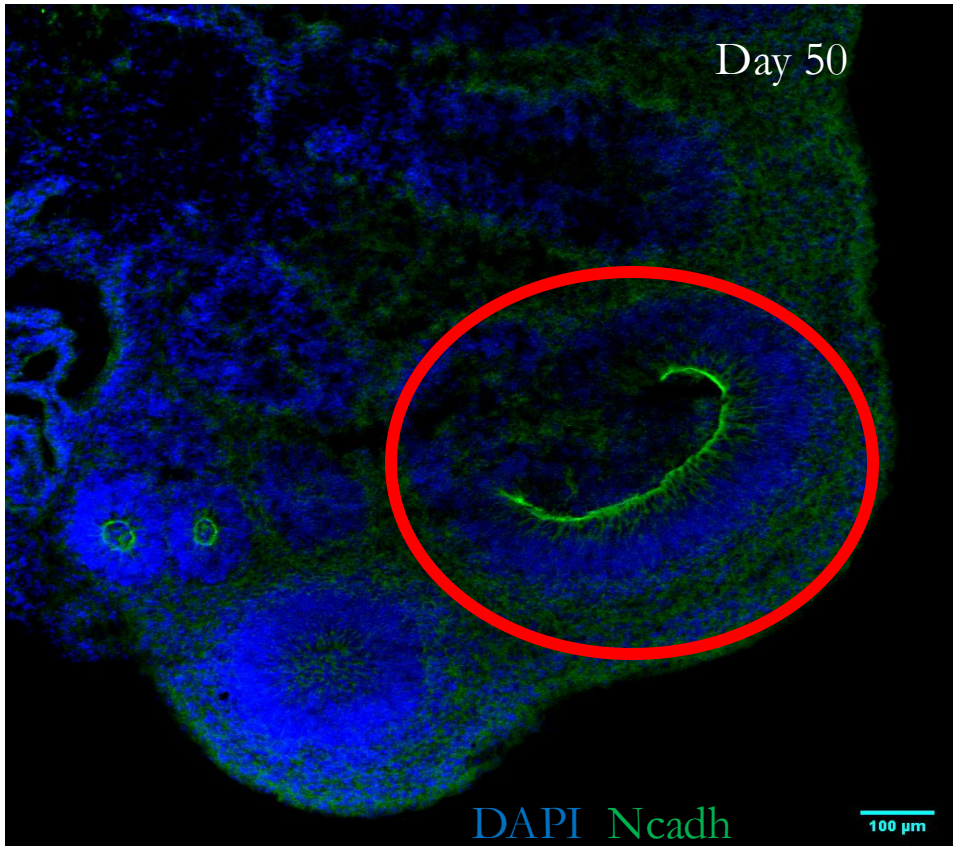


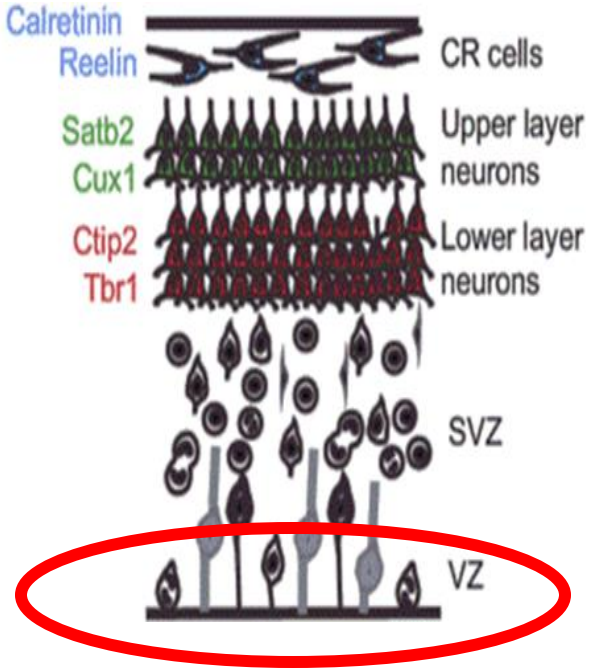
Whole brain organoids



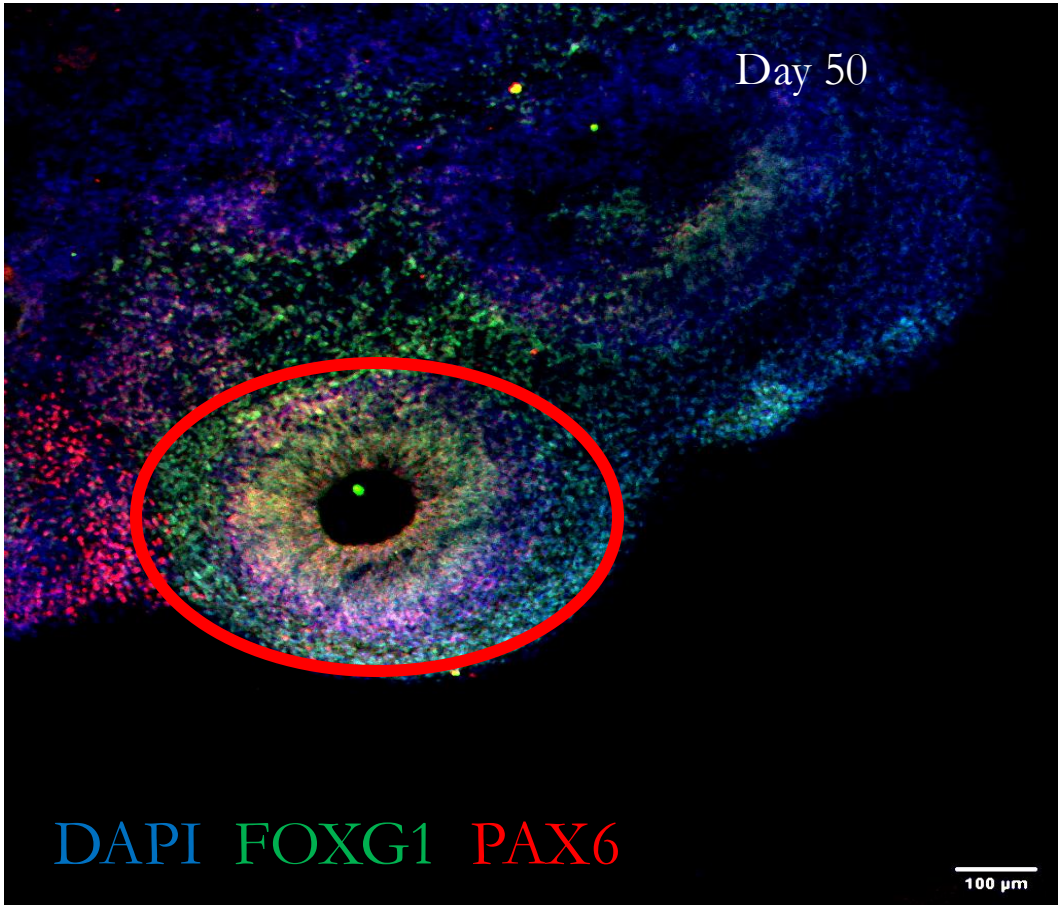
doi: 10.1038/nprot.2014.158

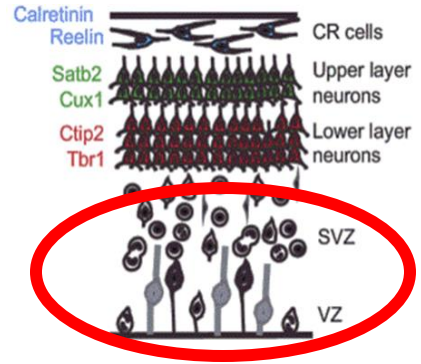
Generation of human cortical organoids

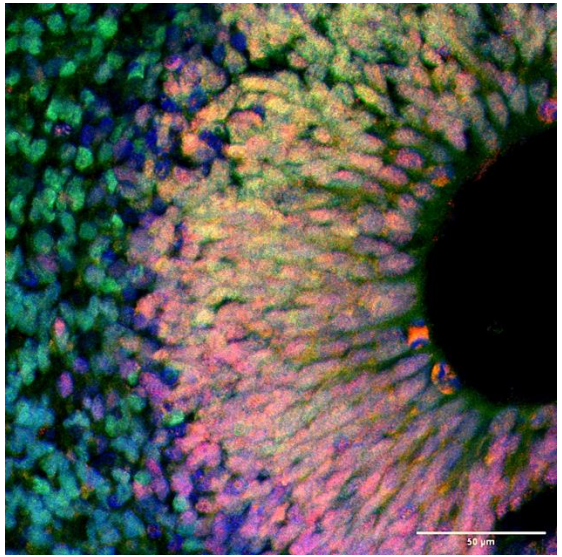




Generation of human cortical organoids

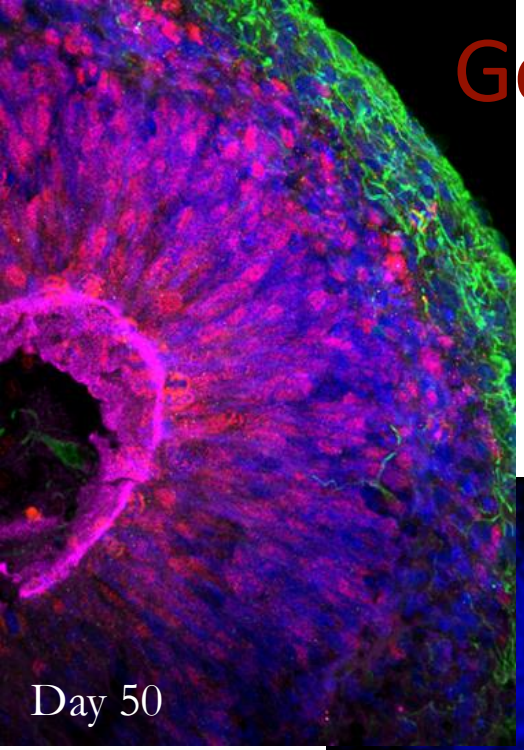


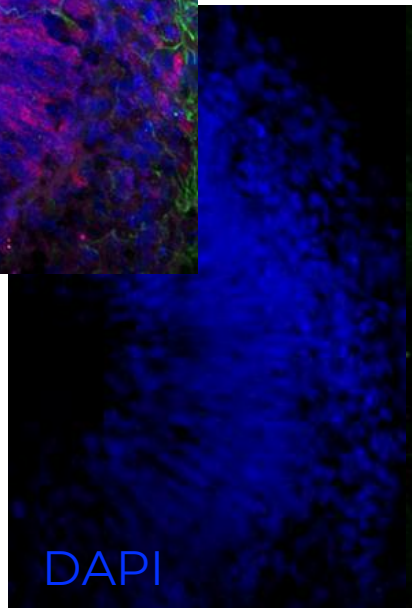


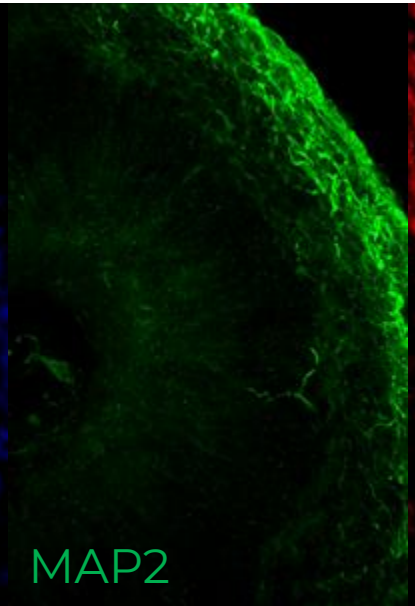


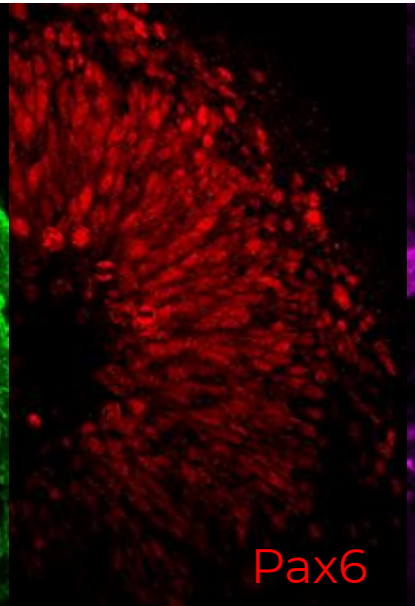
Generation of human cortical organoi

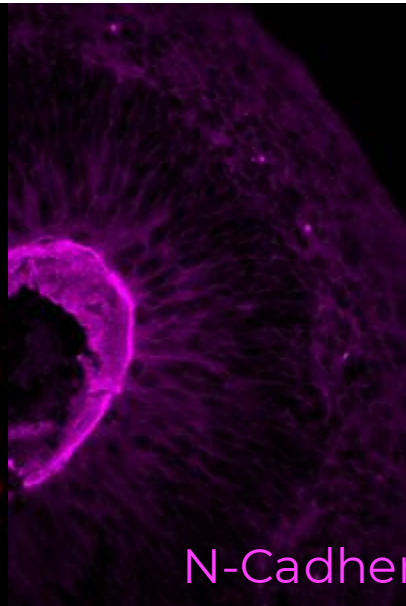
ventricle-like structure - ventricular and subventricular regions





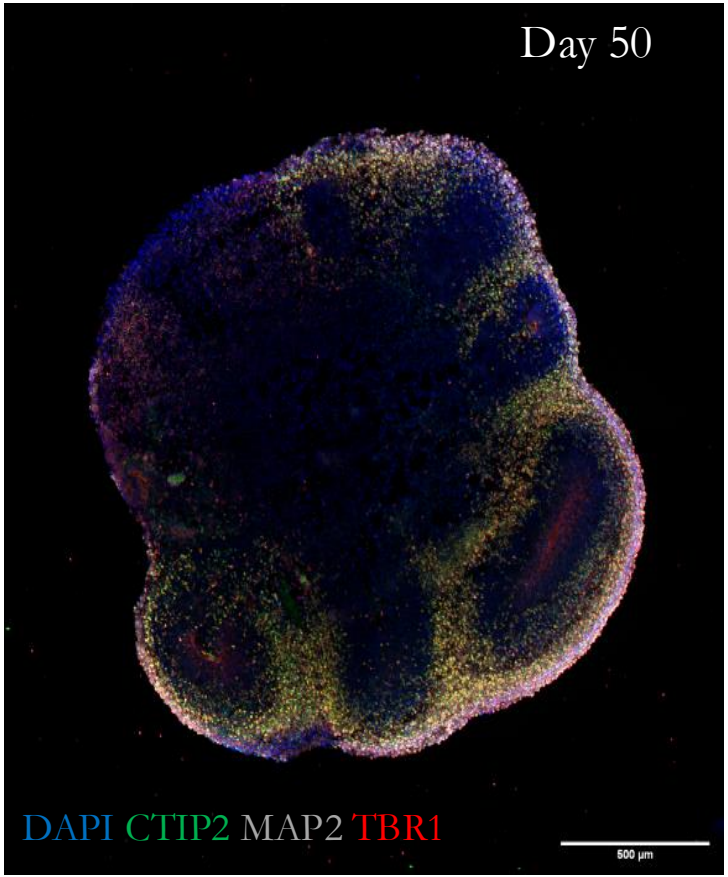


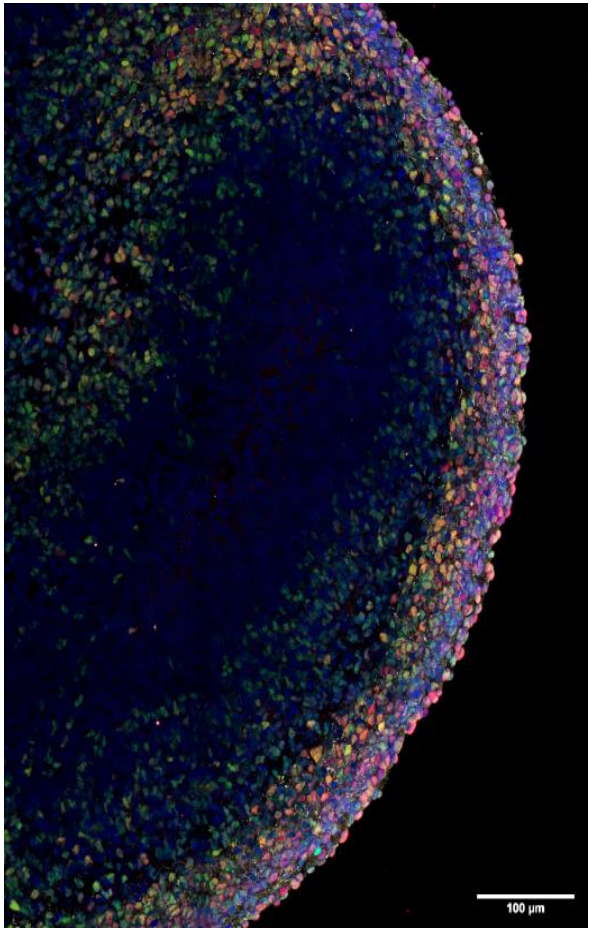


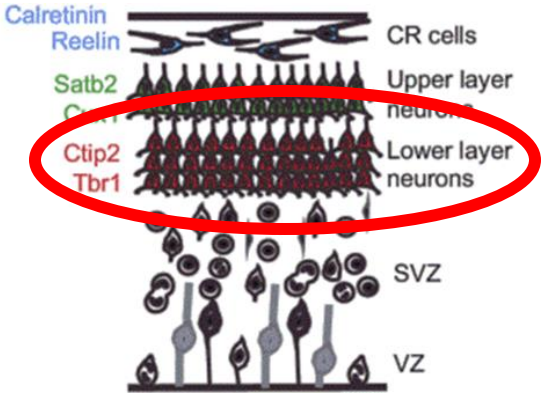


40x

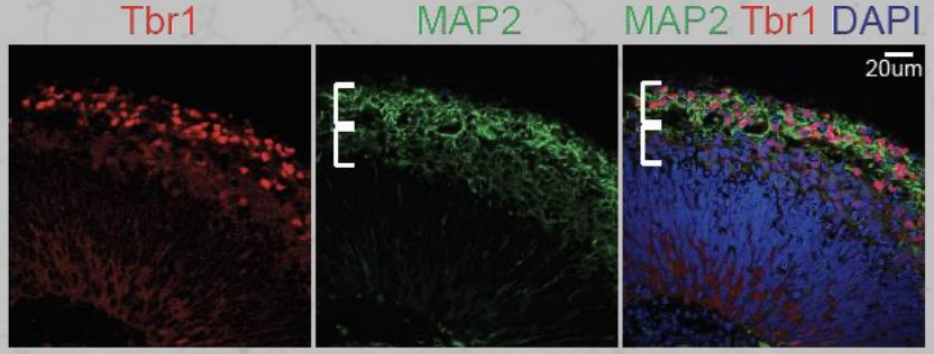
Generation of human cortical organoids

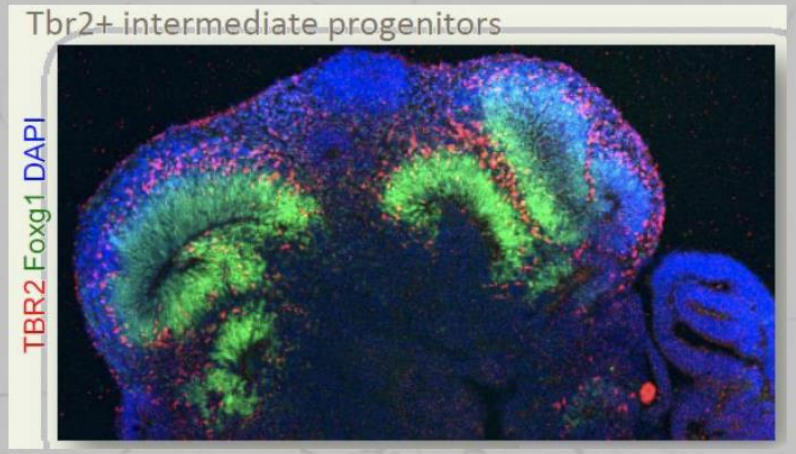


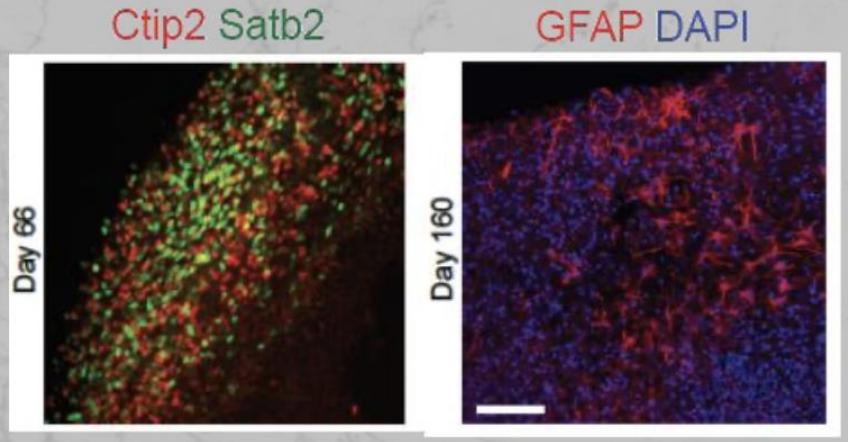


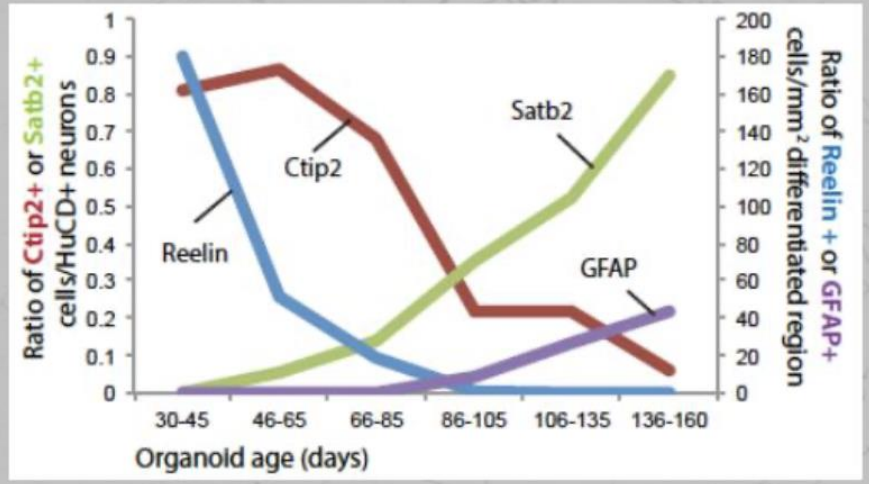


Whole brain organoids





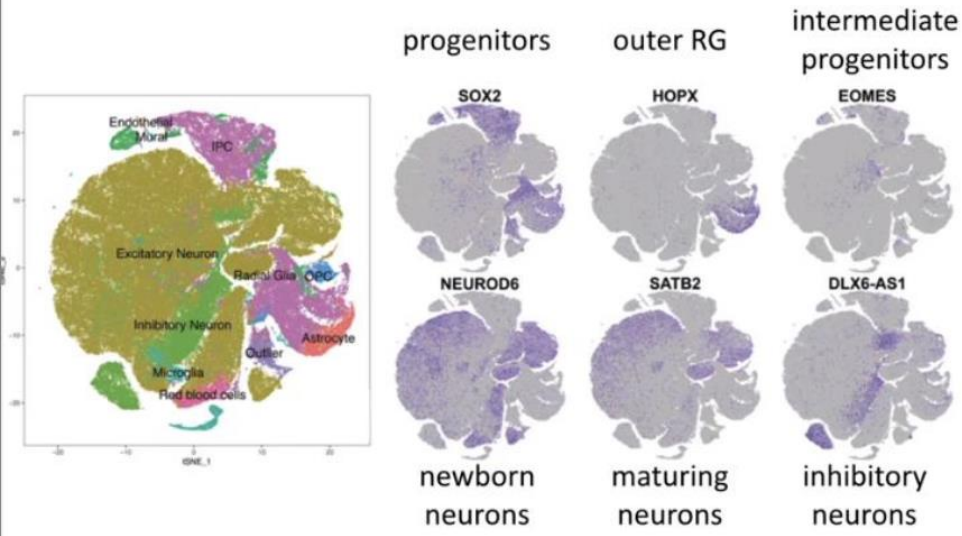




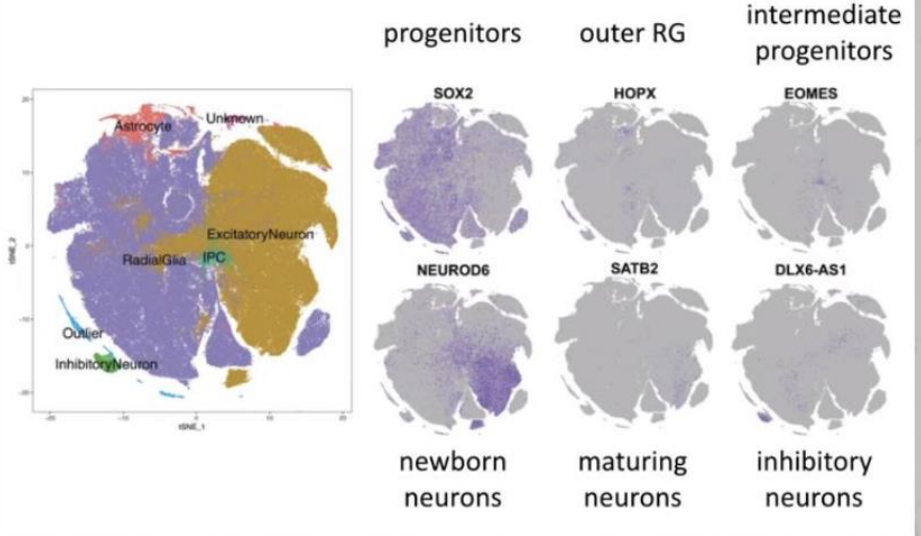
doi: 10.1038/nprot.2014.158

Whole brain organoids

GW6-22, 5 individuals, 7 cortical areas, 189.000 cells

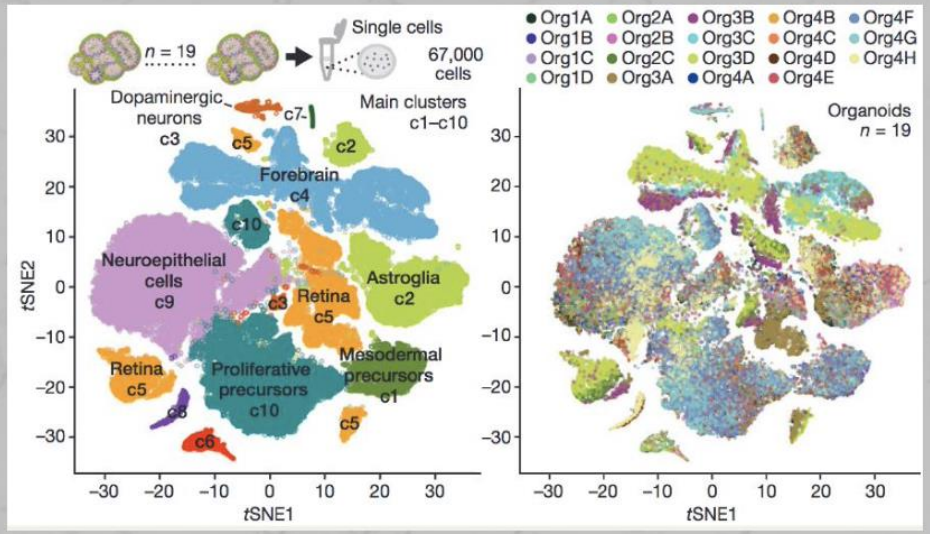


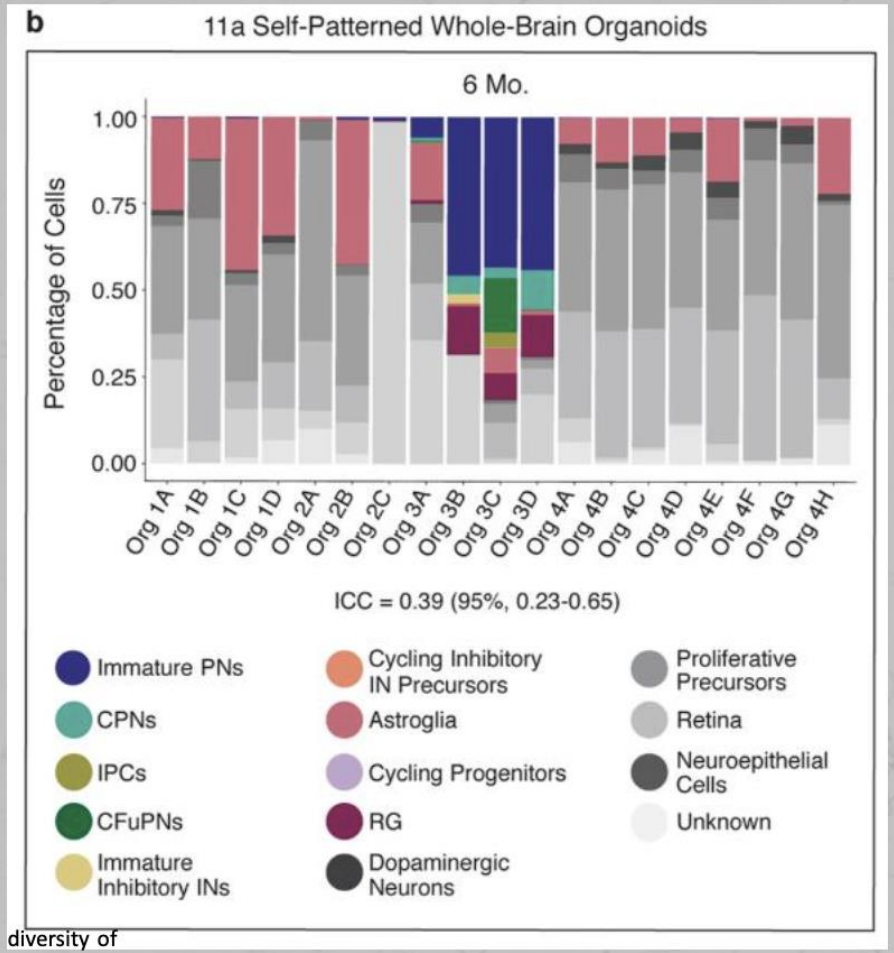
Weeks 3-10, 4 cell lines, 3 protocols,, 109.000 cells



Bhaduri, A., Andrews, M.G., Mancia Leon, W. *et al.* Cell stress in cortical organoids impairs molecular subtype specification. *Nature* 578, 142–148 (2020). https://doi.org/10.1038/s41586-020-1962-0

Whole brain organoids

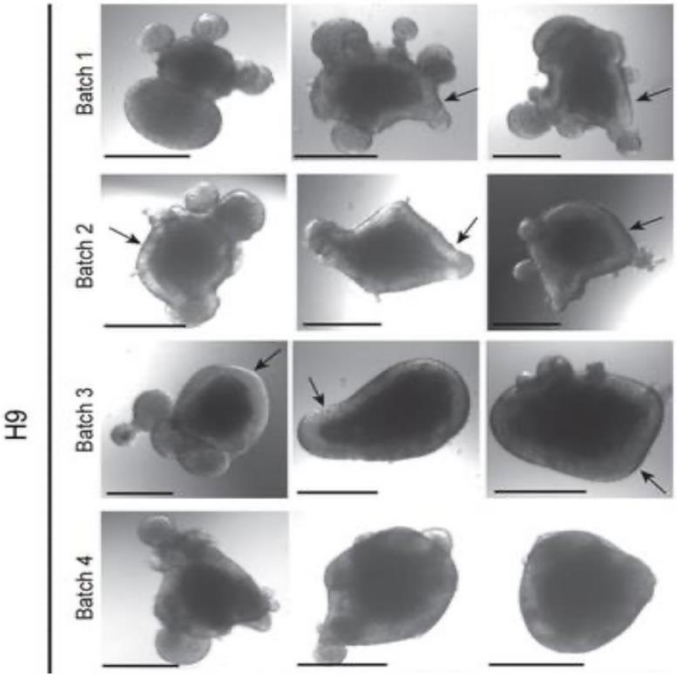


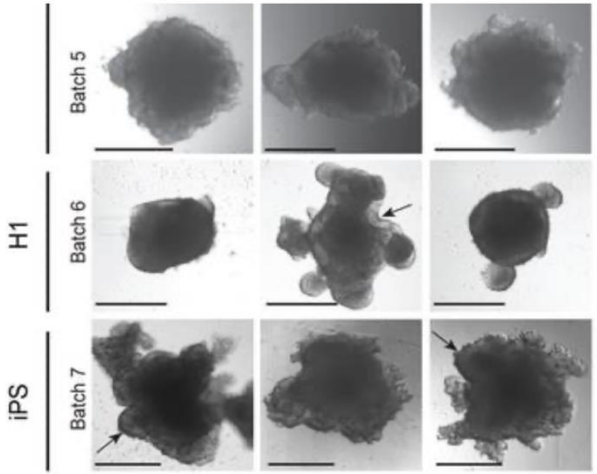


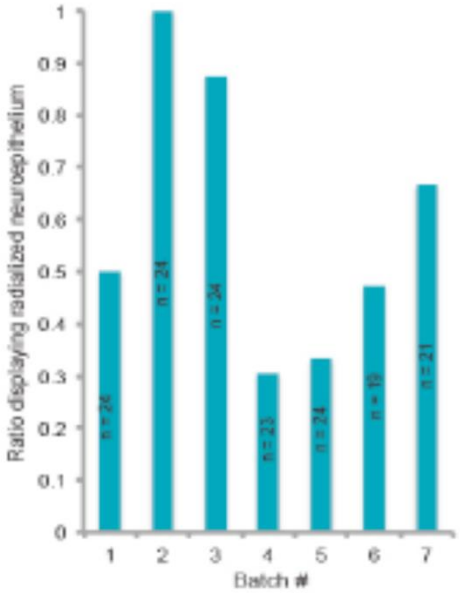
Velasco, S., Kedaigle, A.J., Simmons, S.K. *et al.* Individual brain organoids reproducibly form cell diversity of the human cerebral cortex. *Nature* 570, 523–527 (2019). https://doi.org/10.1038/s41586-019-1289-x

ARE human cortical organoids A RELIABLE MODEL?

THE BATCH SYNDROME







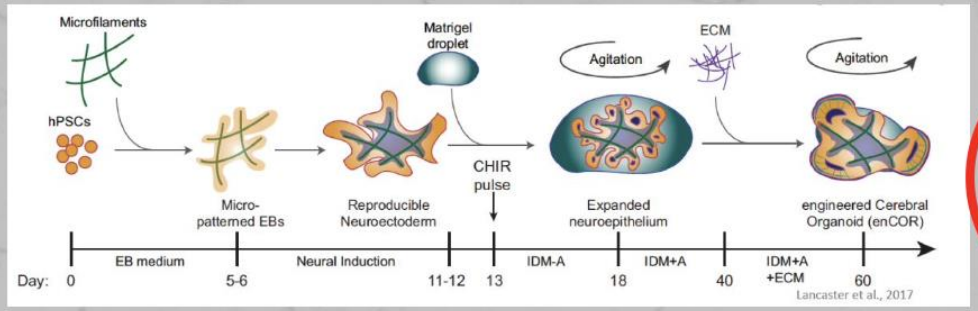
Variable efficiency of neural ectoderm formation.

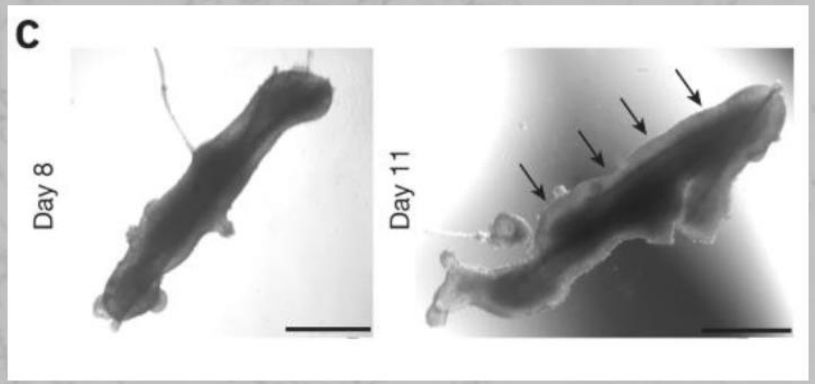
POSSIBLE SOLUTIONS:

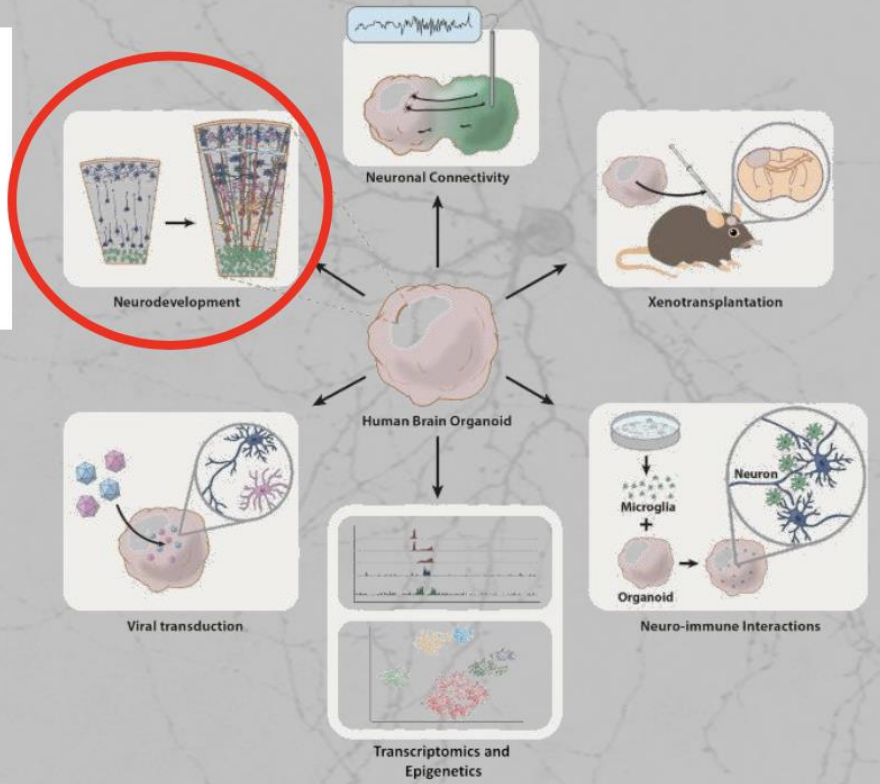
ENGINEERED CEREBRAL ORGANOIDS (ENCORS)

3D BIOPRINTED CONSTRUCTS

Further improvements

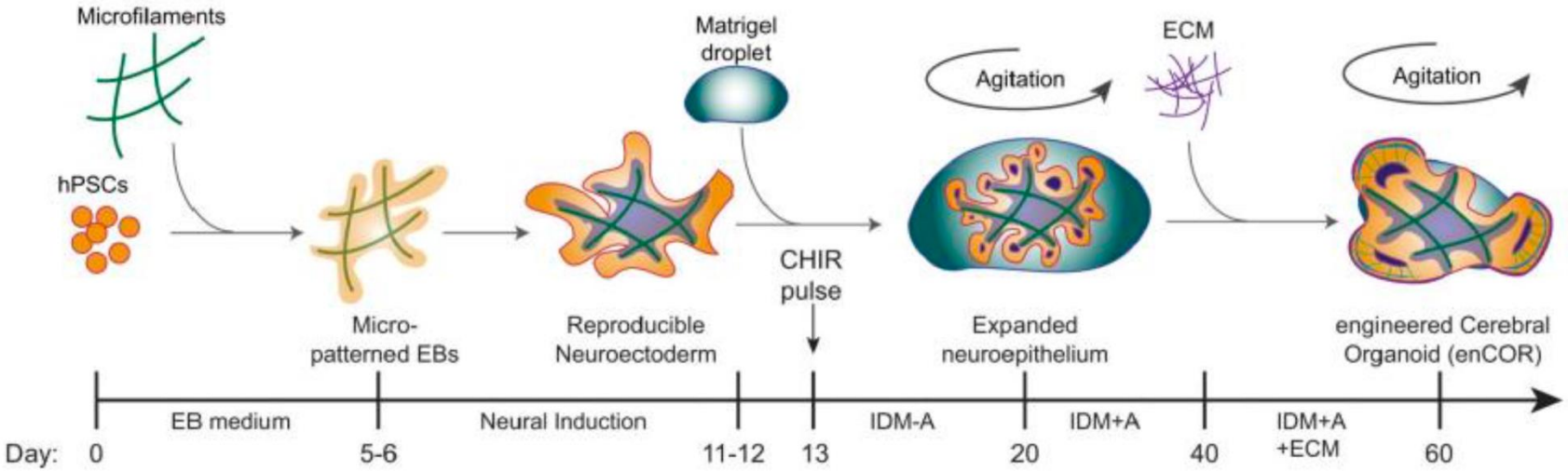




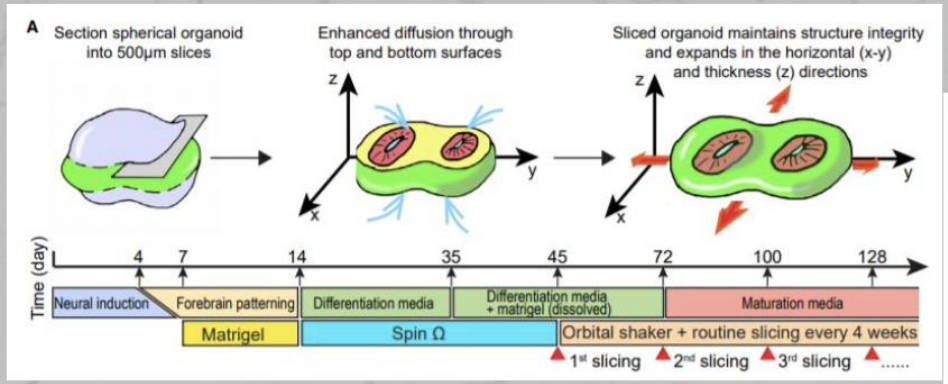


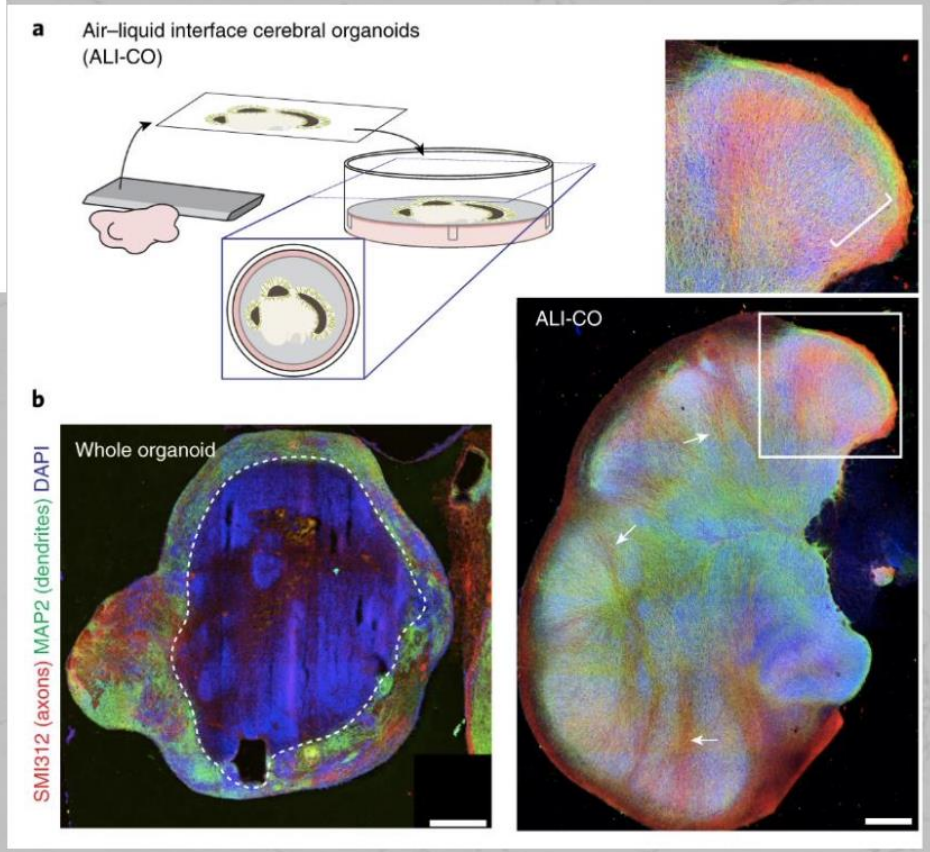
doi: 10.1038/nbt.3906

ENGINEERED CEREBRAL ORGANOIDS (ENCORS)

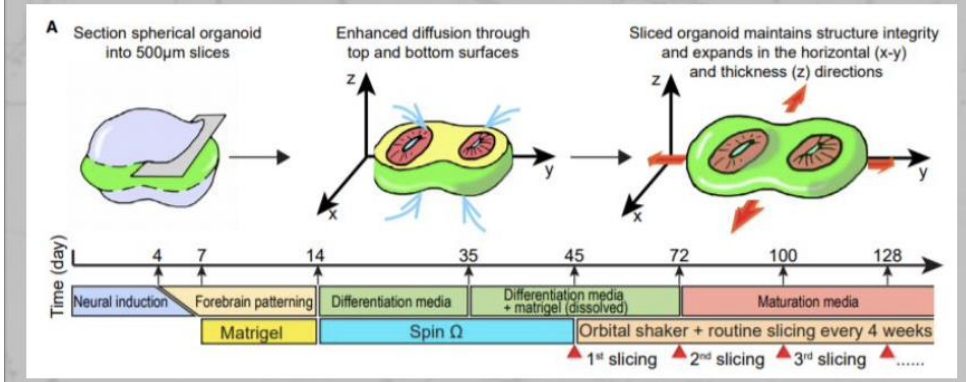


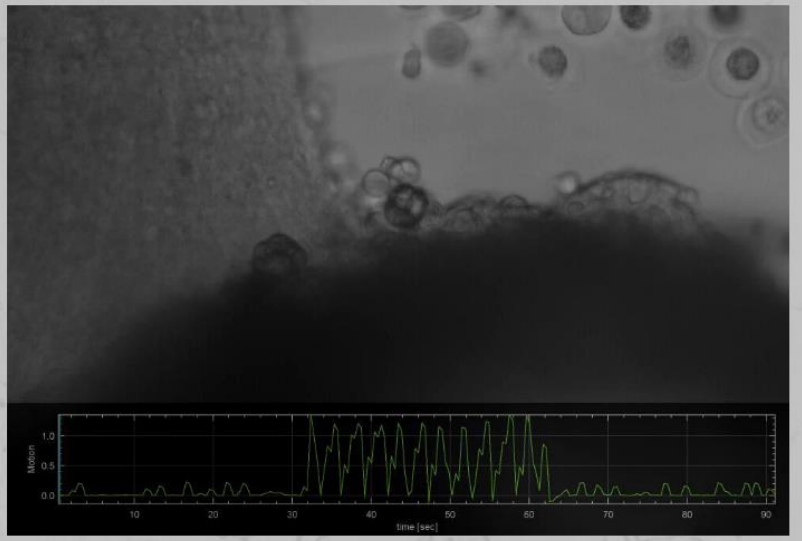
This would allow for the formation of larger tissues with increased surface area to volume ratio.

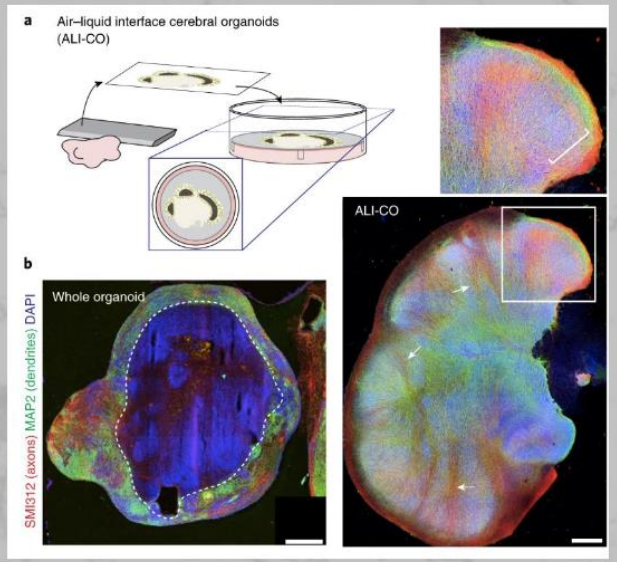


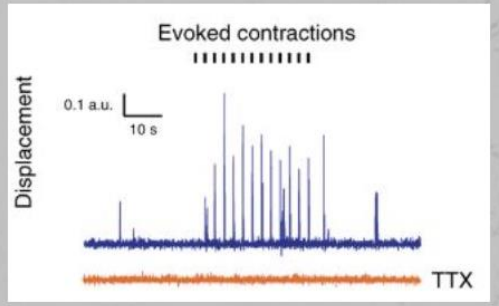


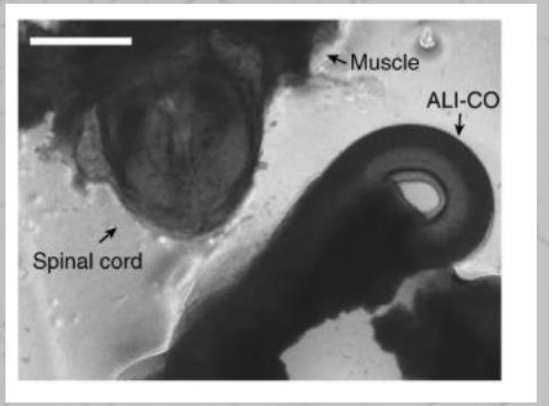
Nat Neurosci. 2019 April ; 22(4): 669–679. doi:10.1038/s41593-019-0350-2





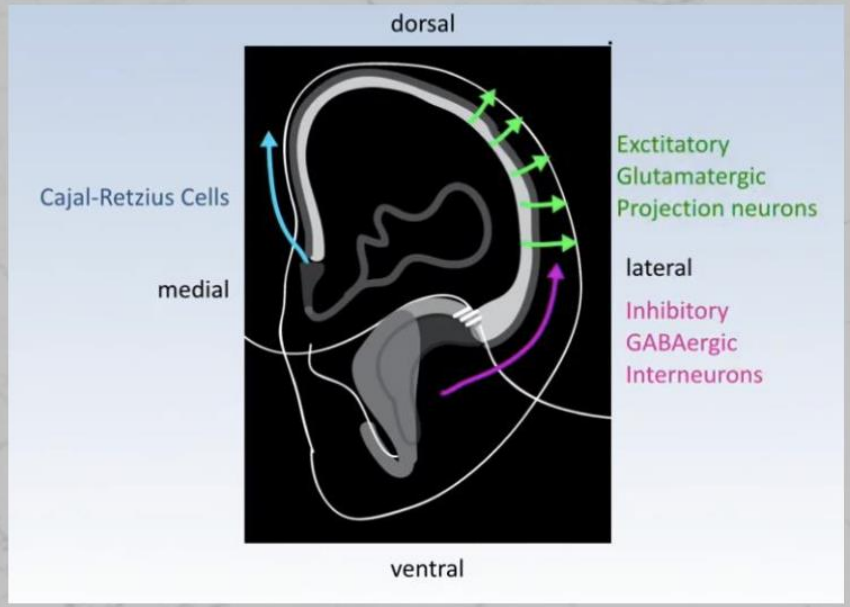


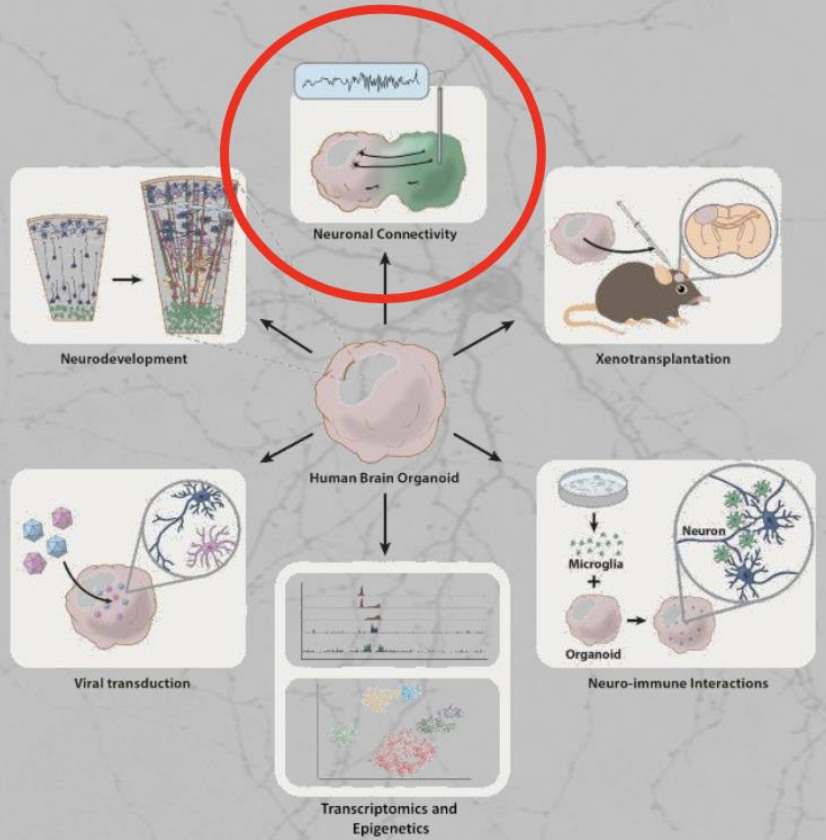




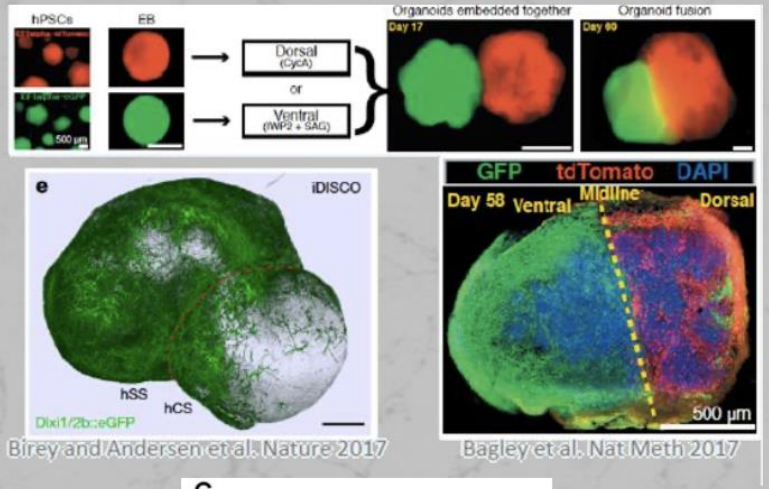
Nat Neurosci. 2019 April ; 22(4): 669–679. doi:10.1038/s41593-019-0350-2

Further improvements



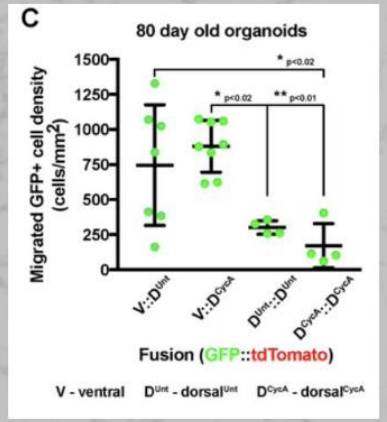


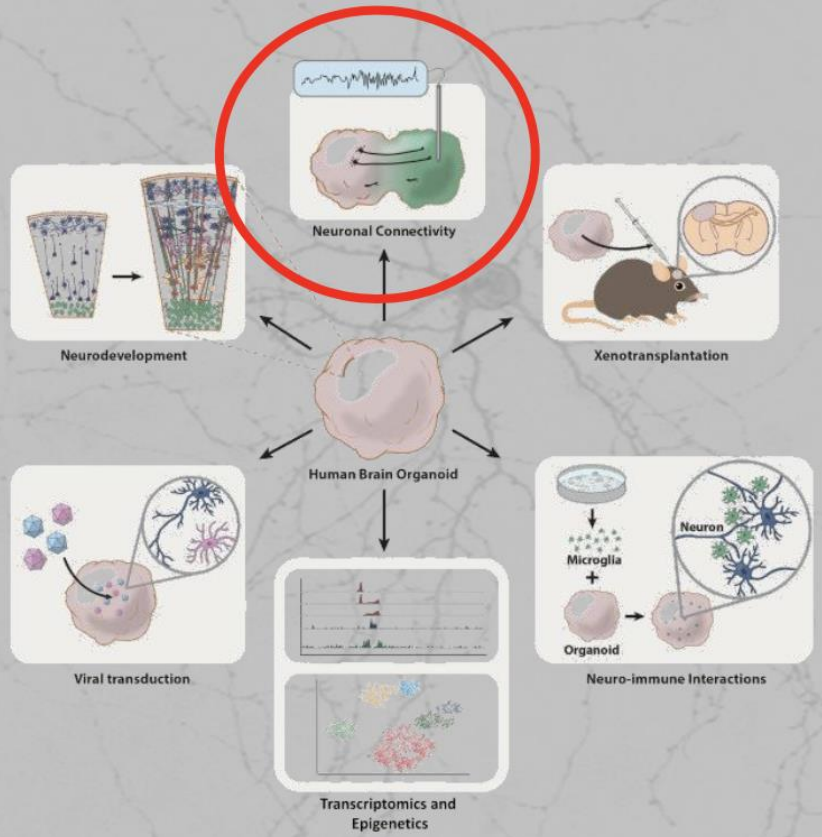
Further improvements



Birey and Andersen et al. Nature 2017

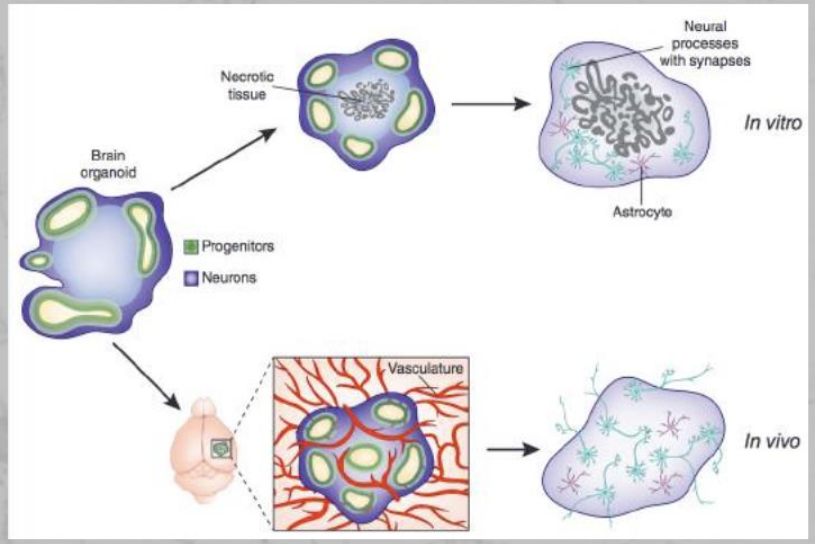
Bagley et al. Nat Meth 2017

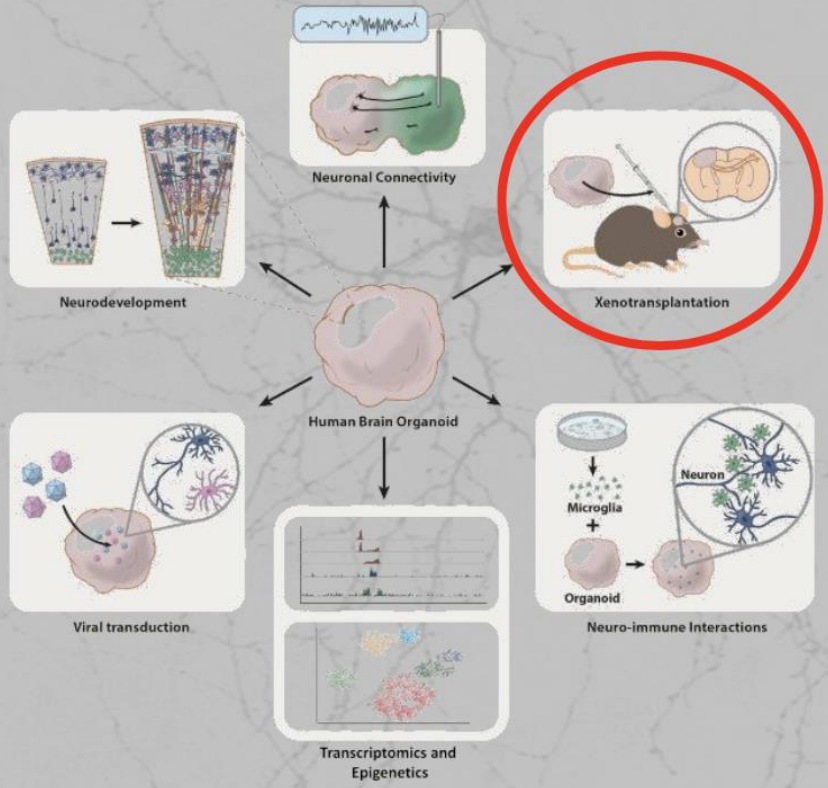




doi: 10.1038/nmeth.4304

Further improvements





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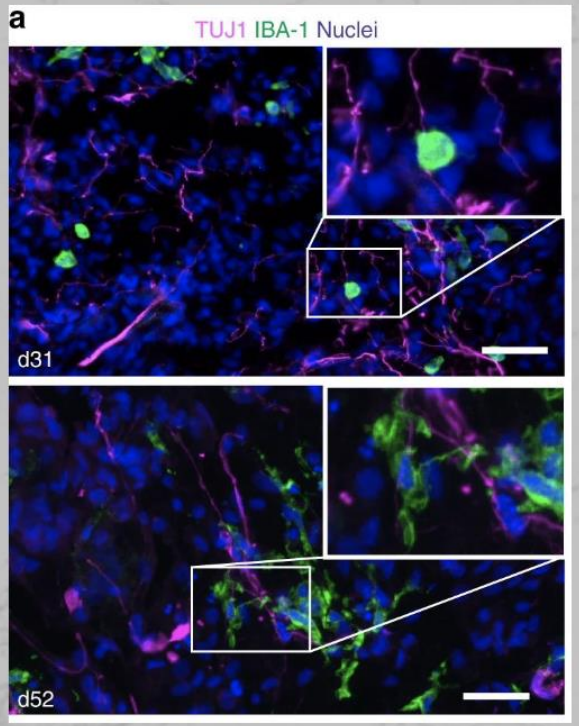
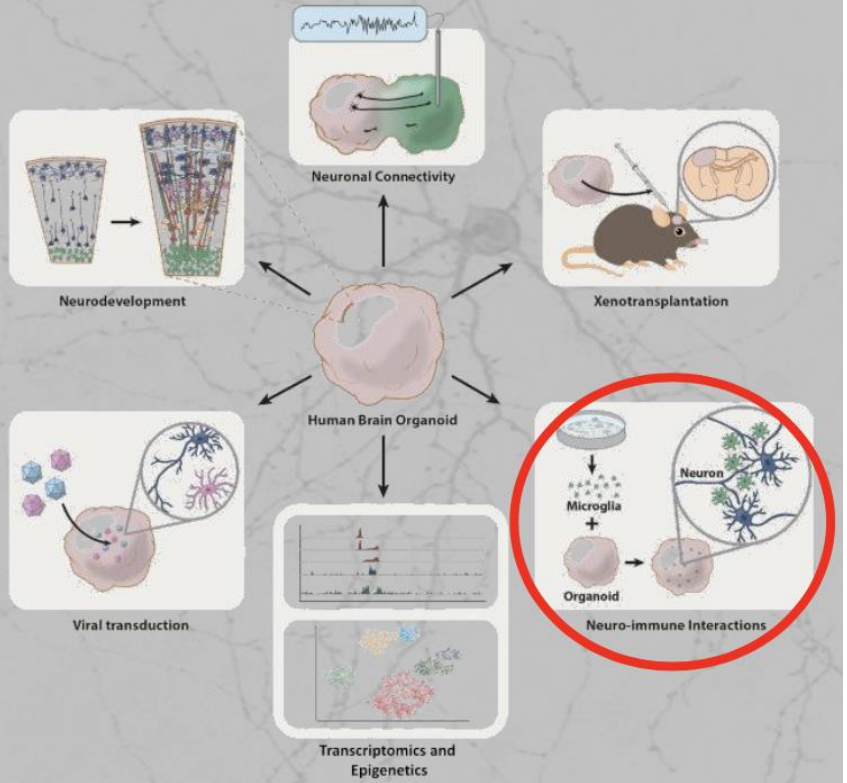
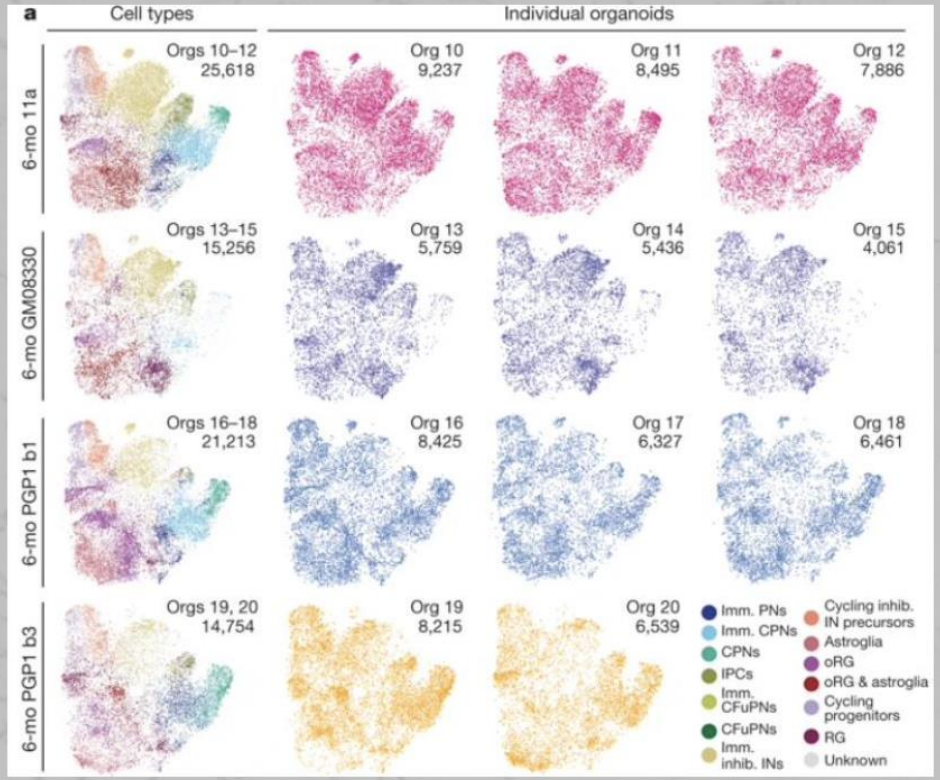
Further improvements

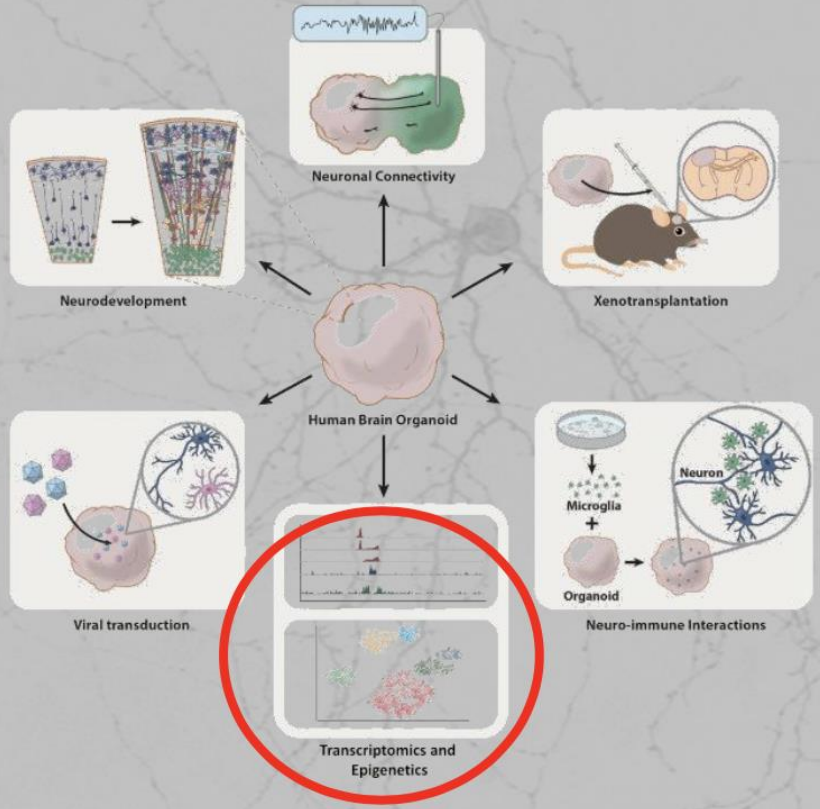




Ormel, P.R., Vieira de Sá, R., van Bodegraven, E.J. *et al.* Microglia innately develop within cerebral organoids. *Nat Commun* 9, 4167 (2018). https://doi.org/10.1038/s41467-018-06684-2

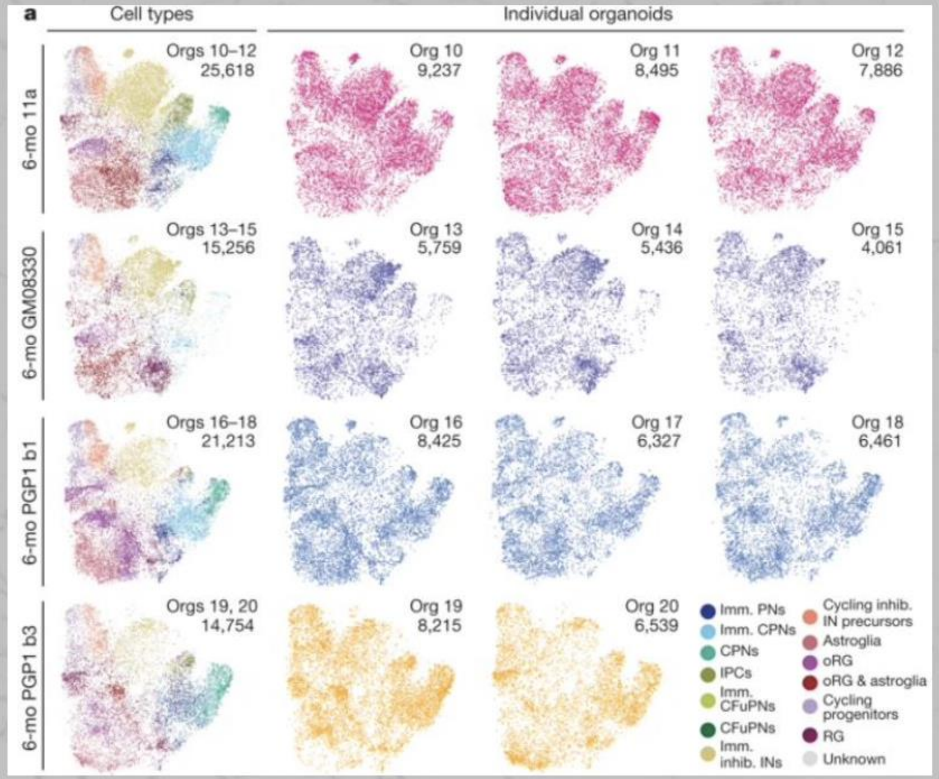
Further improvements

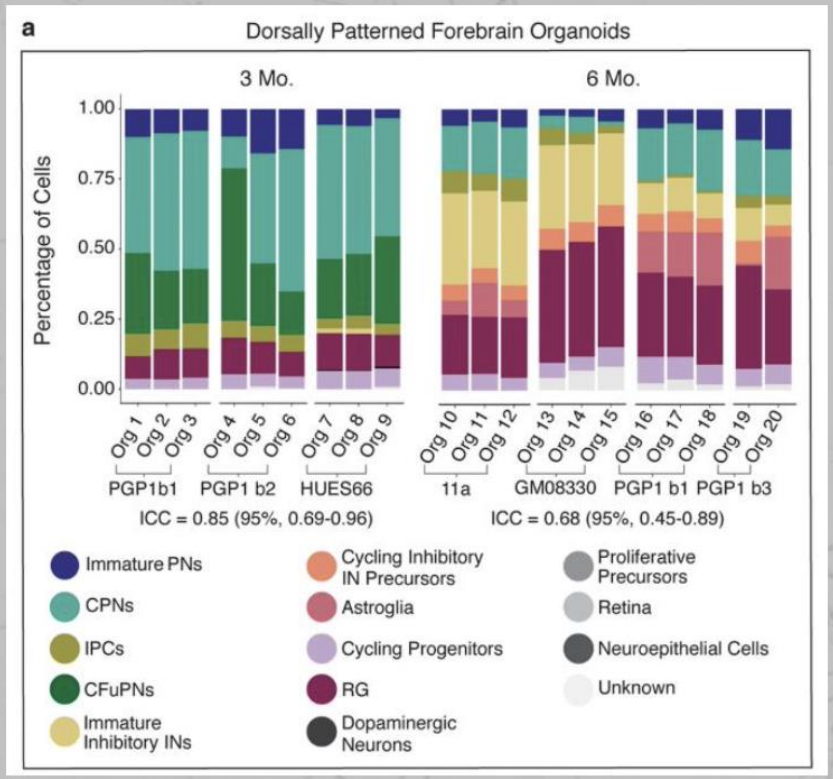




Velasco, S., Kedaigle, A.J., Simmons, S.K. *et al.* Individual brain organoids reproducibly form cell diversity of the human cerebral cortex. *Nature* 570, 523–527 (2019). https://doi.org/10.1038/s41586-019-1289-x

Further improvements

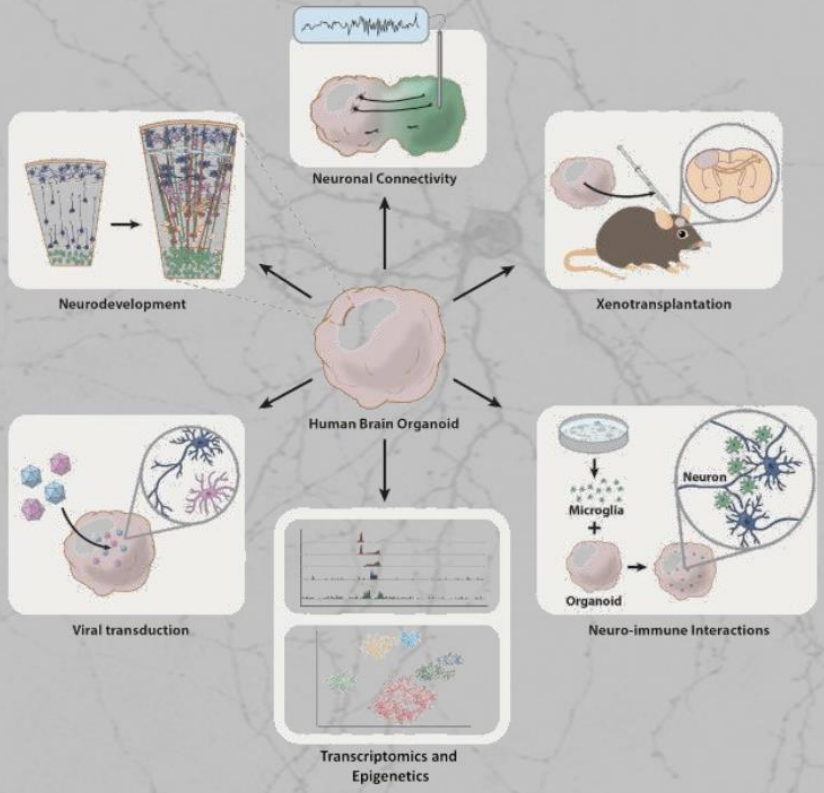




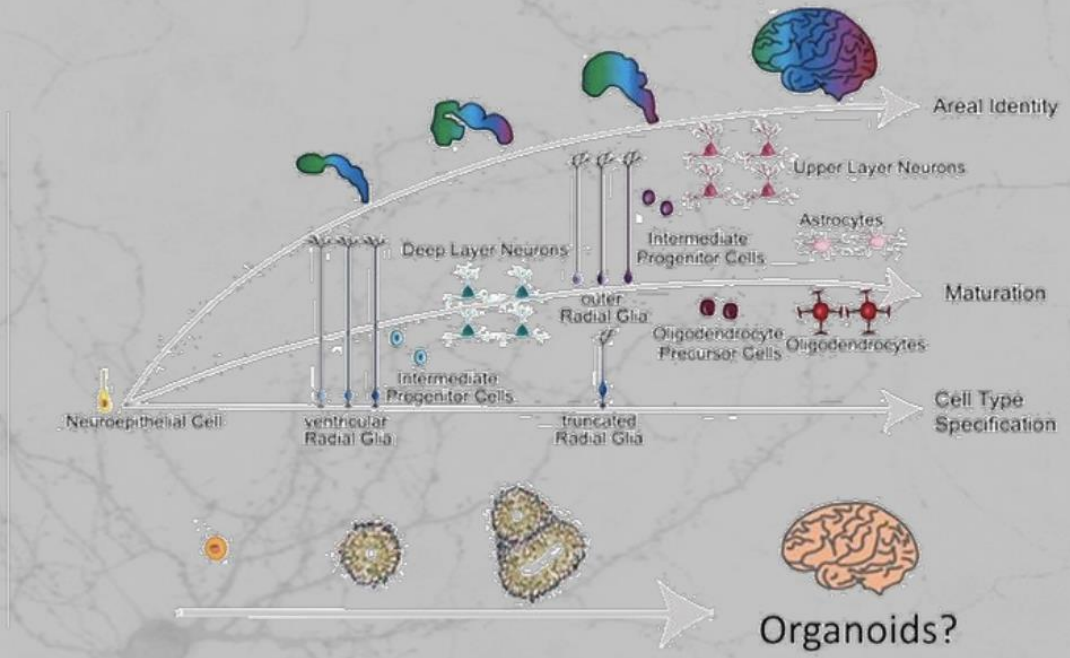
Velasco, S., Kedaigle, A.J., Simmons, S.K. *et al.* Individual brain organoids reproducibly form cell diversity of the human cerebral cortex. *Nature* 570, 523–527 (2019). https://doi.org/10.1038/s41586-019-1289-x

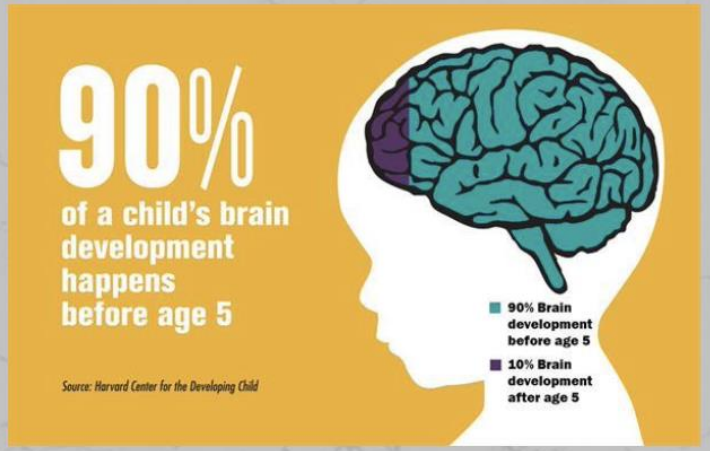
Summary

Strengths	Weakness
Various CNS regions	Lack of a axial patterning
Progenitor zone and early neuronal layering	No maturation and proper layering over time
Outer Radial Glia	Lack of other cell types (vasculature, immune..)
Astrocytes and oligodendrocytes	No reliable proportion in cell type generation
Neuronal connectivity and functional networking	No reliable transcriptional signature



Neurodevelopmental disorders





Microcephaly

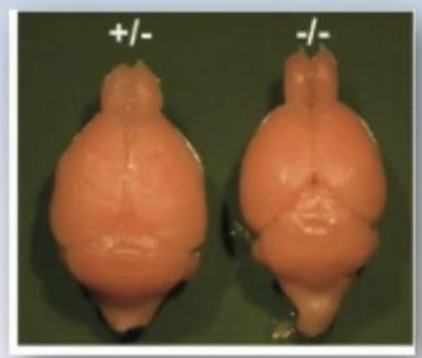
Cerebral organoids model human brain development and microcephaly

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[1], Andrew P. Jackson[2] & Juergen A. Knoblich[1]

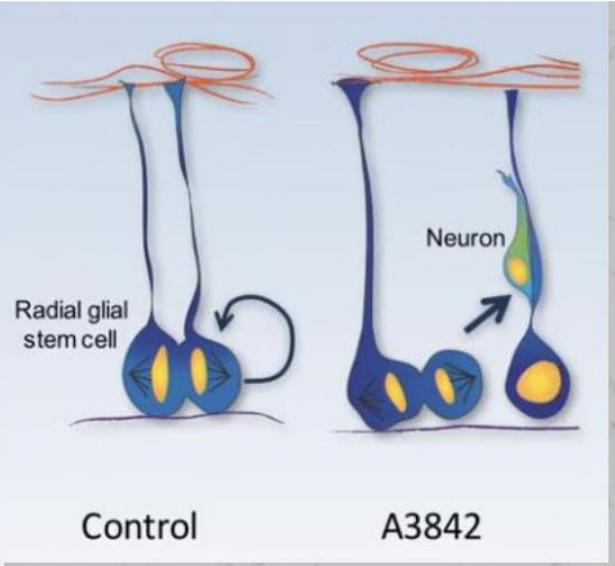
Human microcephaly (Nde1)

Alkuraya et al. Am J Hum Genet. 2011.

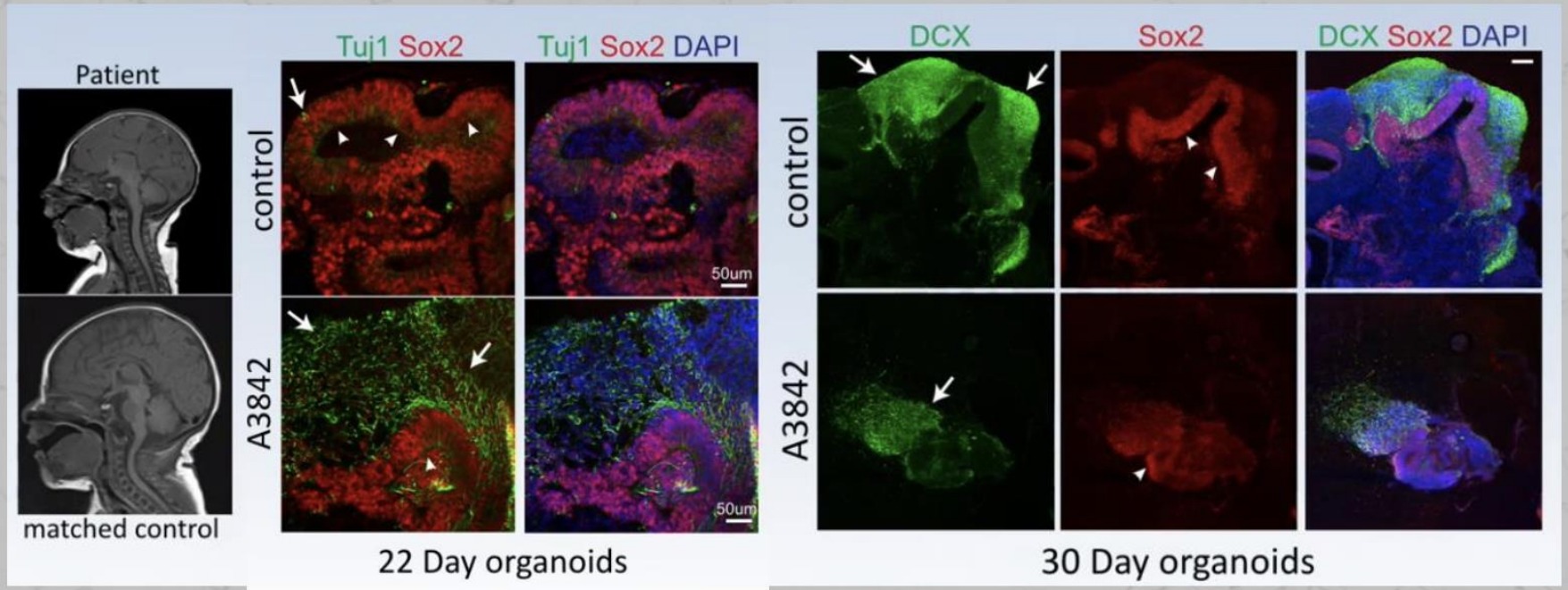
Mouse model (Nde1)

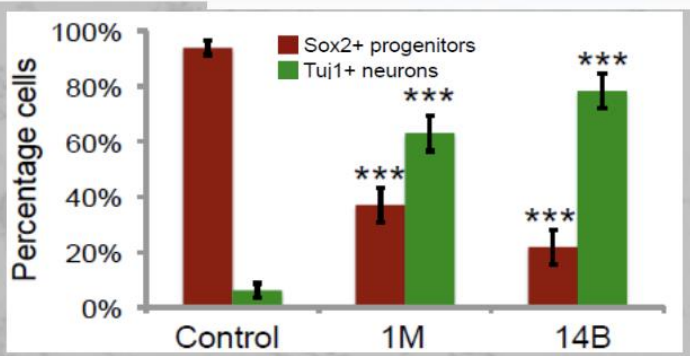


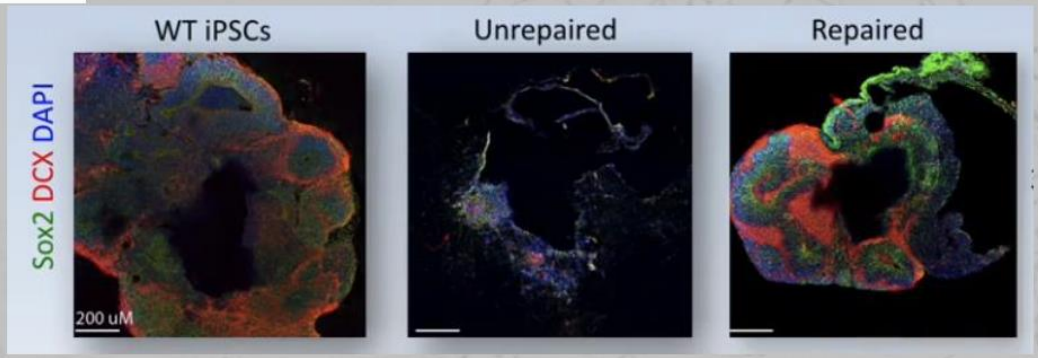
Feng and Walsh. Neuron 2004.



Lancaster, M., Renner, M., Martin, C. *et al.* Cerebral organoids model human brain development and microcephaly. *Nature* 501, 373–379 (2013). https://doi.org/10.1038/nature12517

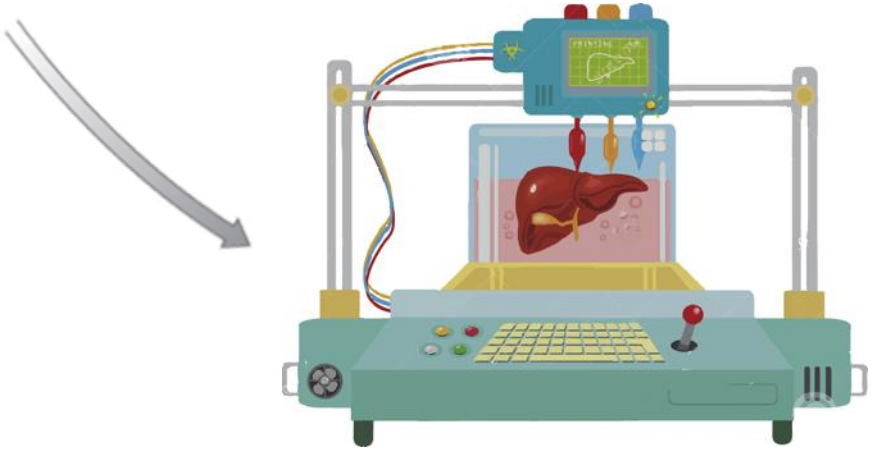

Patient
matched control
Tuj1 Sox2
Tuj1 Sox2 DAPI
control
A3842
50um
50um
22 Day organoids
DCX
Sox2
DCX Sox2 DAPI
control
A3842
30 Day organoids


Percentage cells
100%
80%
60%
40%
20%
0%
Sox2+ progenitors
Tuj1+ neurons
***
***
***
***
Control
1M
14B


WT iPSCs
Unrepaired
Repaired
Sox2 DCX DAPI
200 uM

3D BIOPRINTED CONSTRUCTS

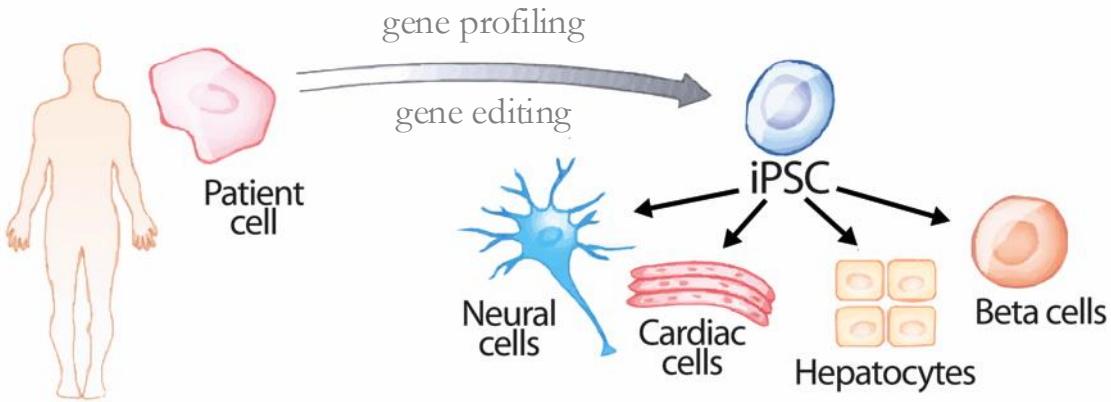
Induced Pluripotent Stem Cells (iPSCs)



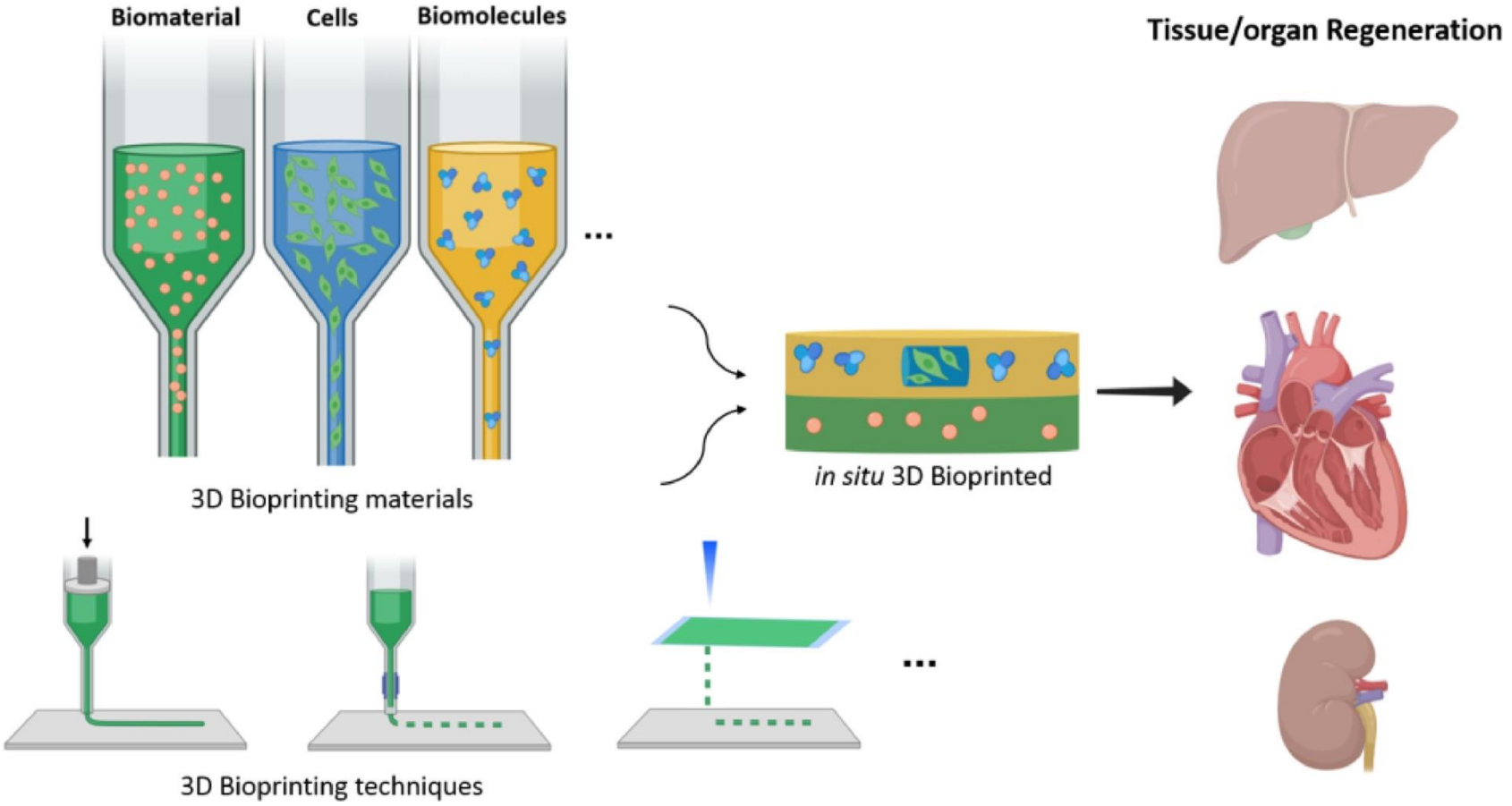
3D Bio-fabrication

bricks

Induced Pluripotent Stem Cells (iPSCs)

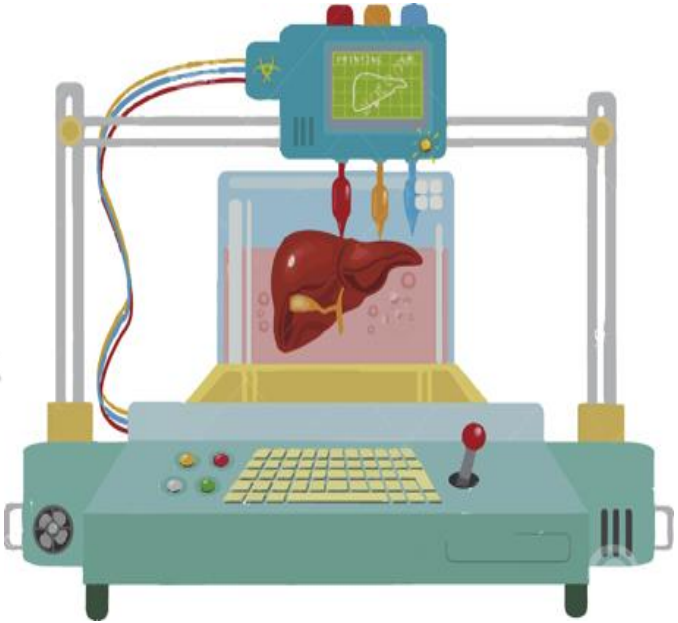


New tools- bricks

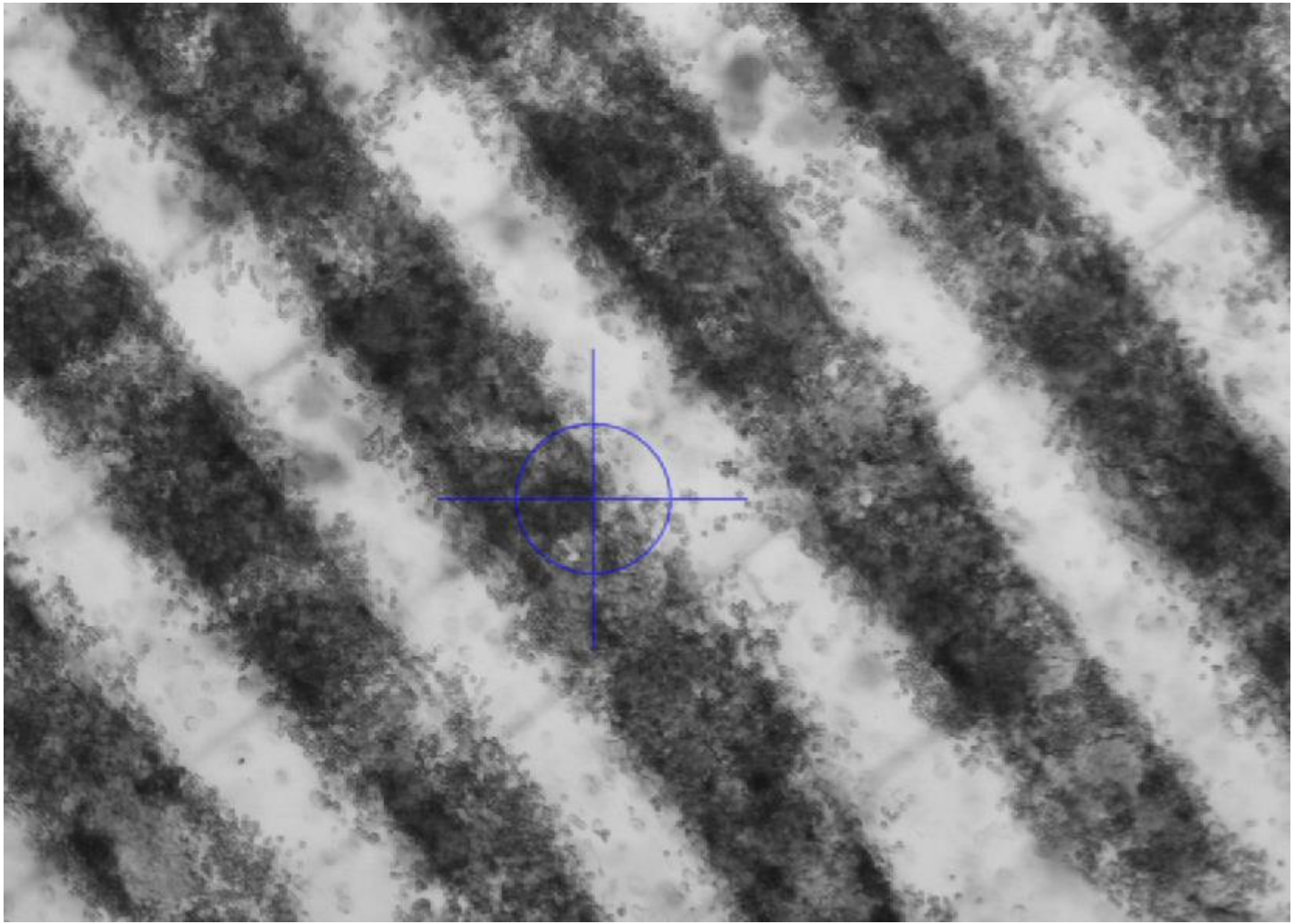


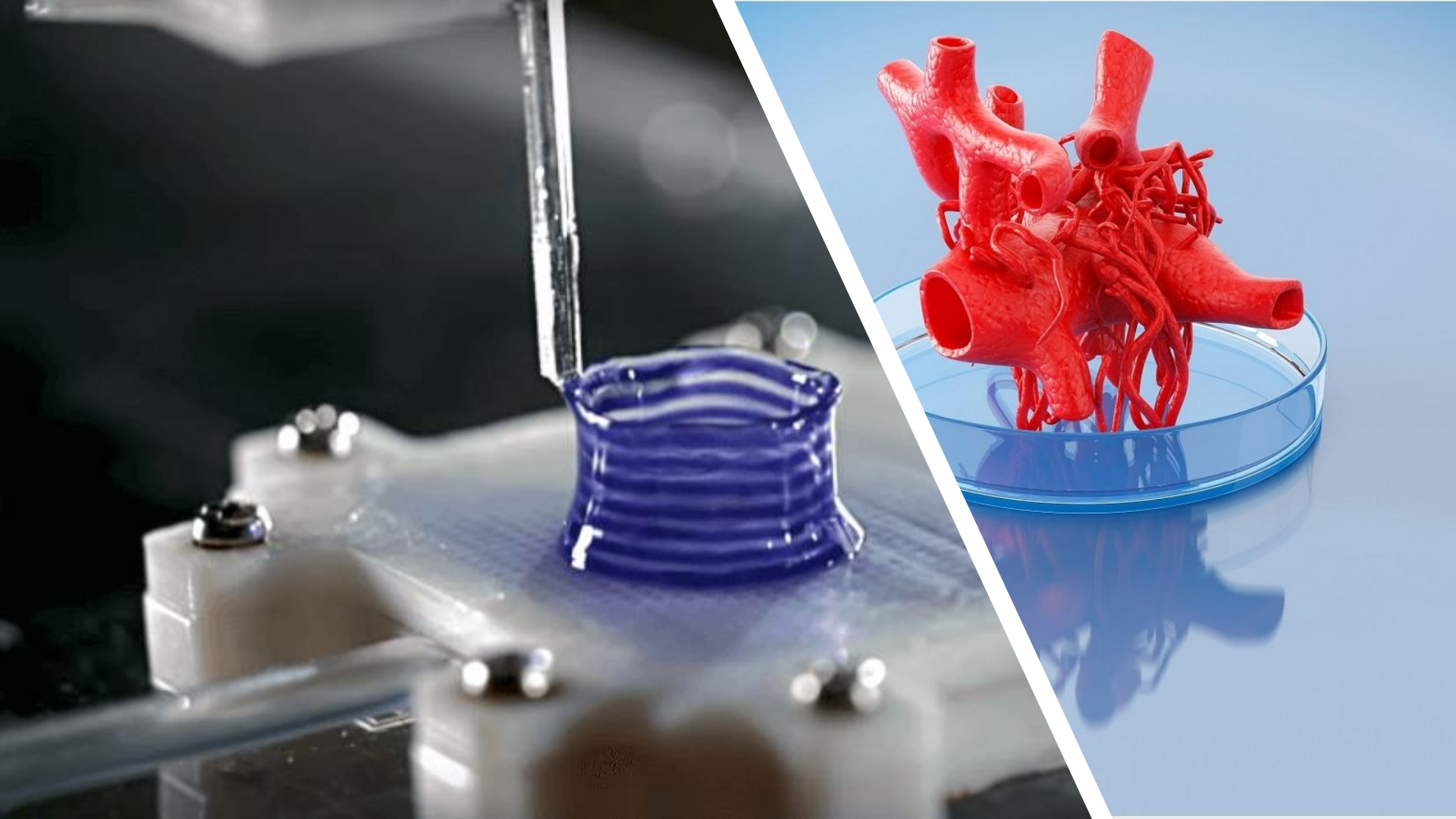
3D Bioprinting

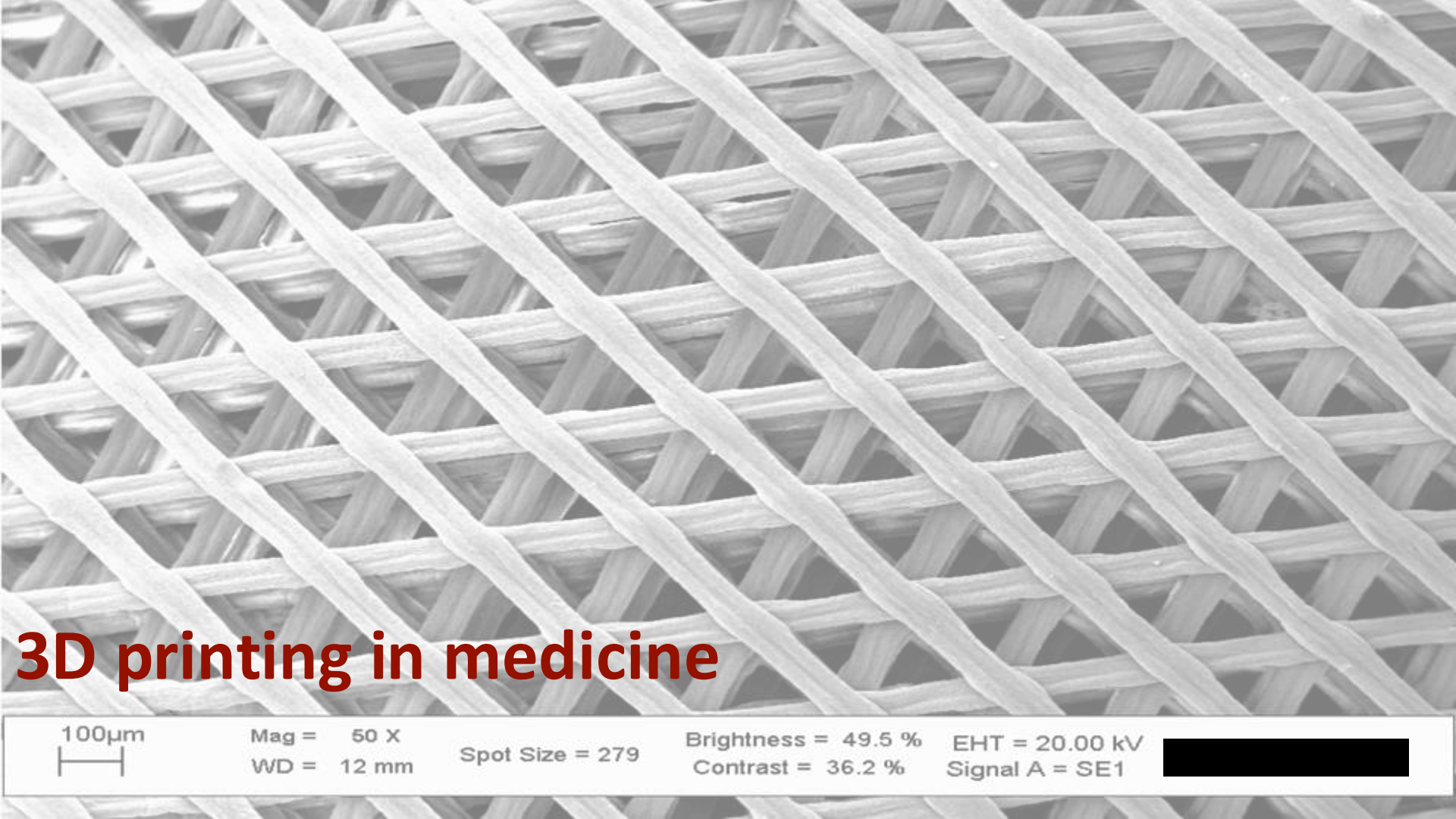
Bioink



3D Bio-fabrication



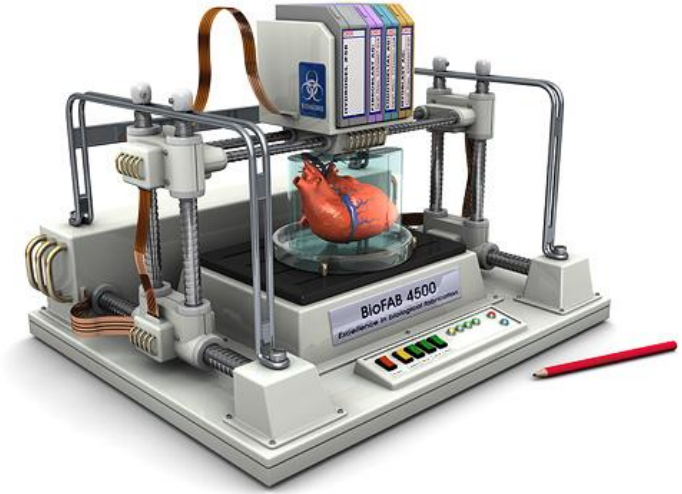




3D printing in medicine

Prosthetic 3D printing

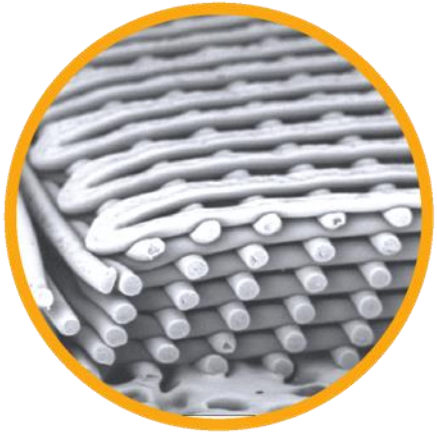
Ehanced reality



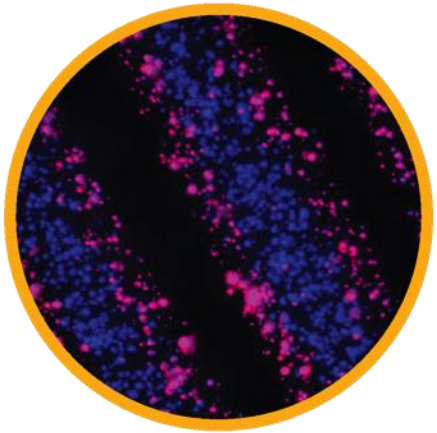
3D BIOPRINTED CONSTRUCTS

Physiologically relevant Human-derived platforms

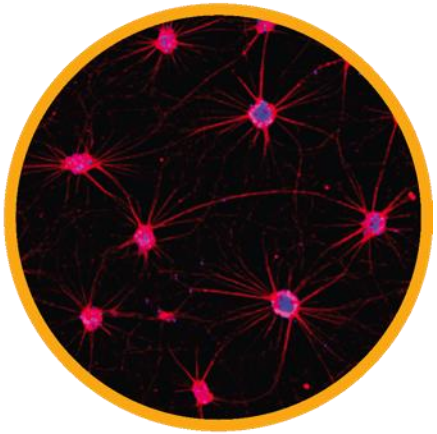
consistent



three-dimensional



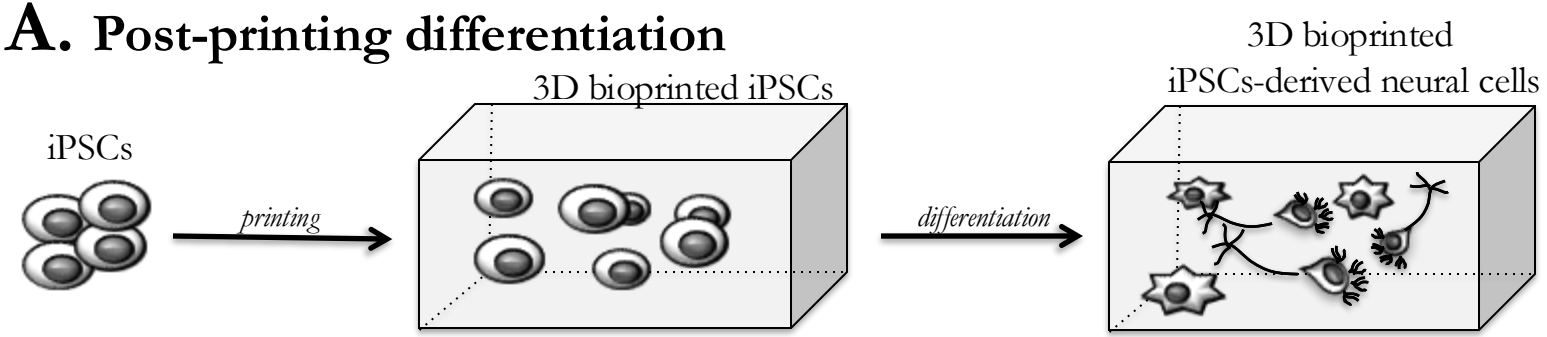
versatile



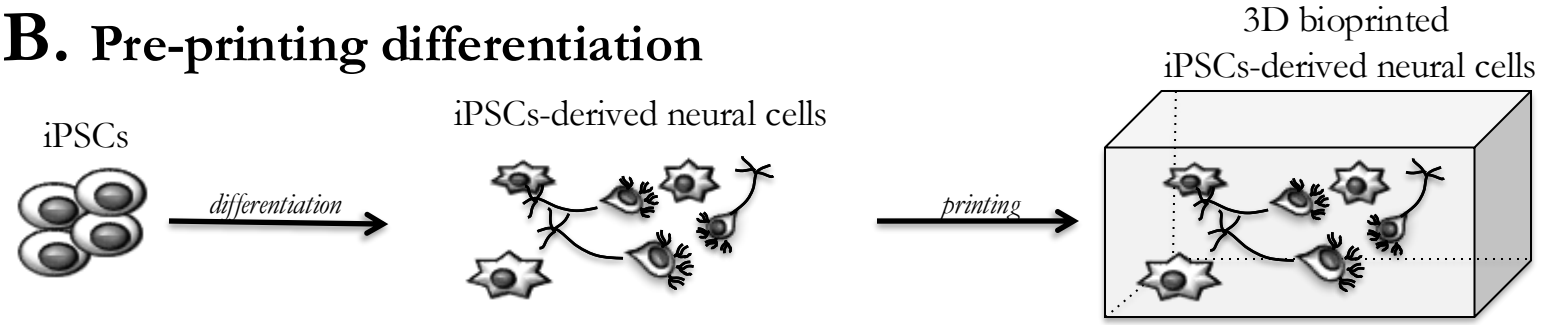
functional

iPSCs Printing Strategies

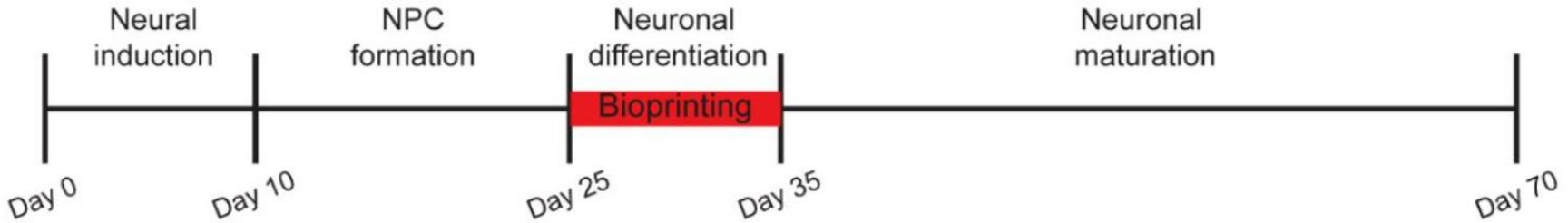
A. Post-printing differentiation

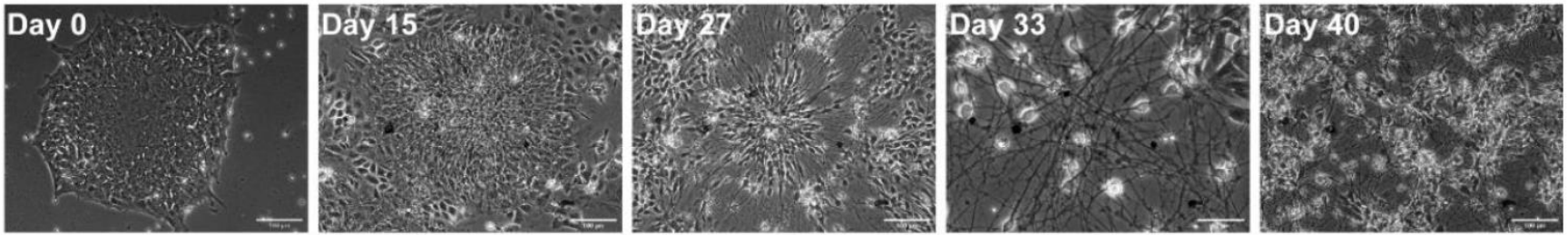


B. Pre-printing differentiation

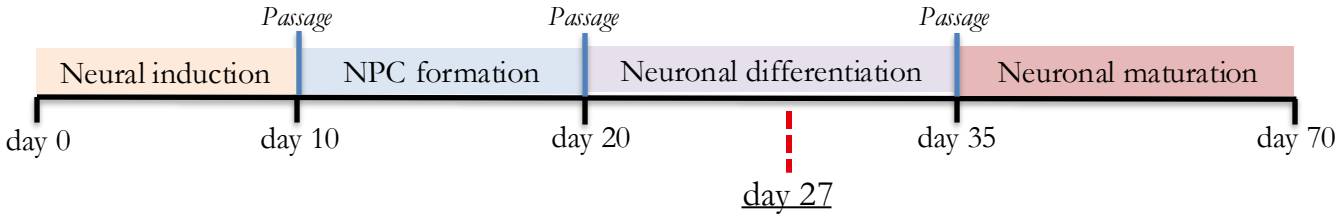


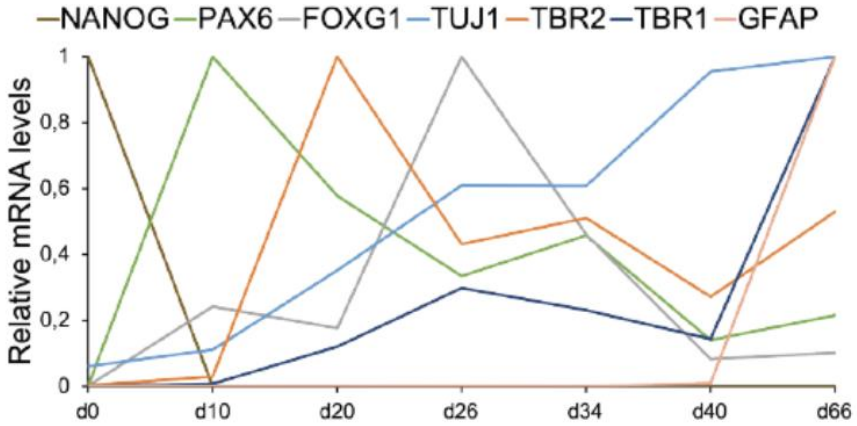
3D bioprinting of differentiating cortical neurons





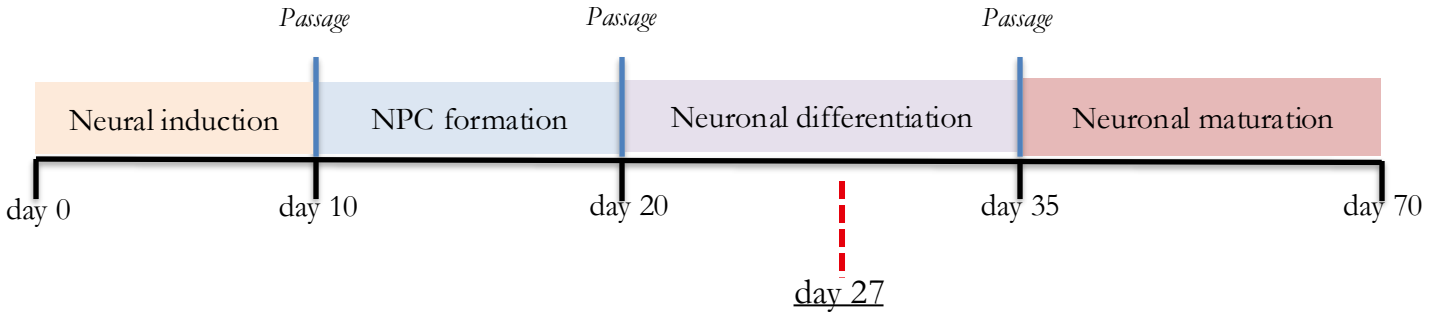
Neural differentiation of human iPSCs

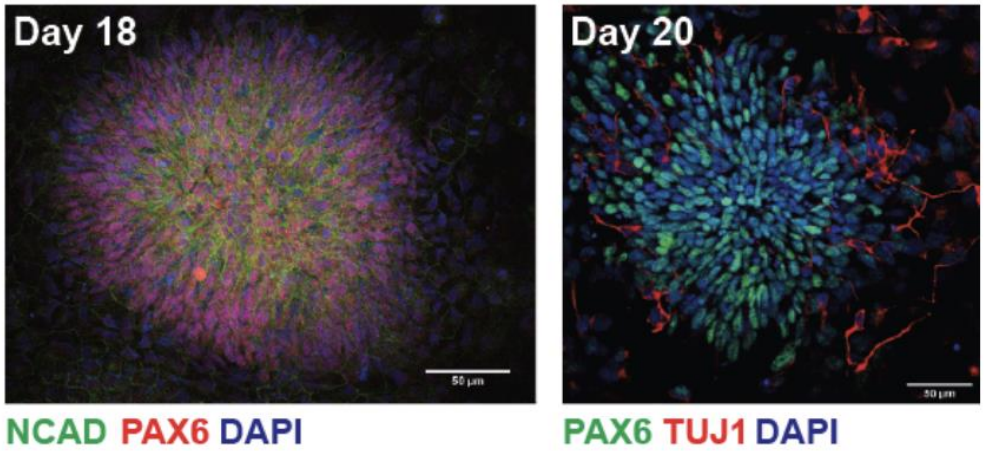




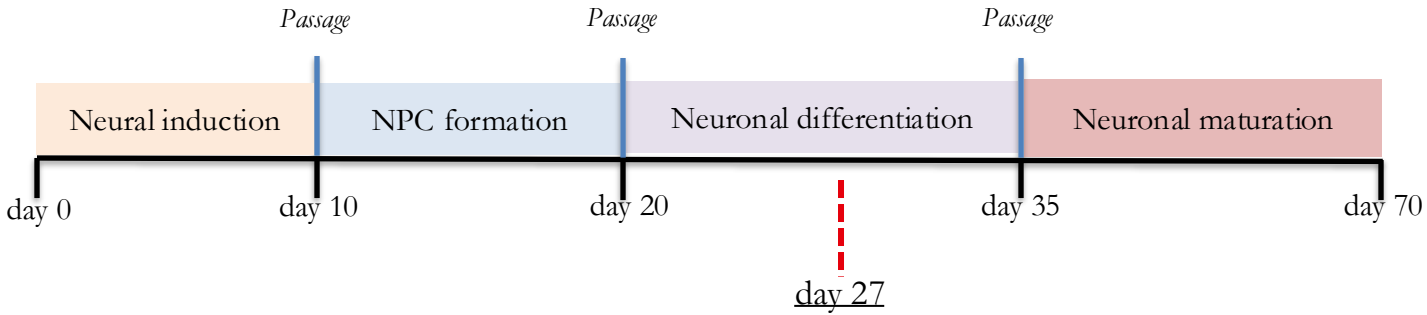
Real-time qRT-PCR analysis during iPSC differentiation.

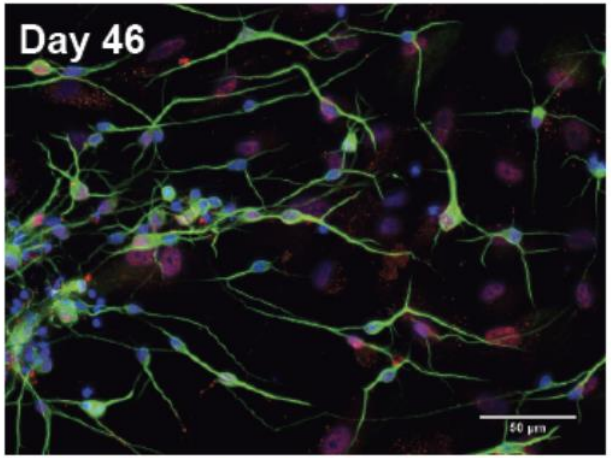
Neural differentiation of human iPSCs



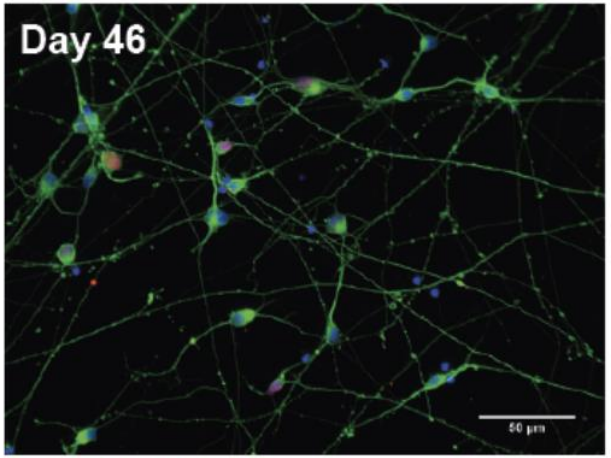


Neural differentiation of human iPSCs

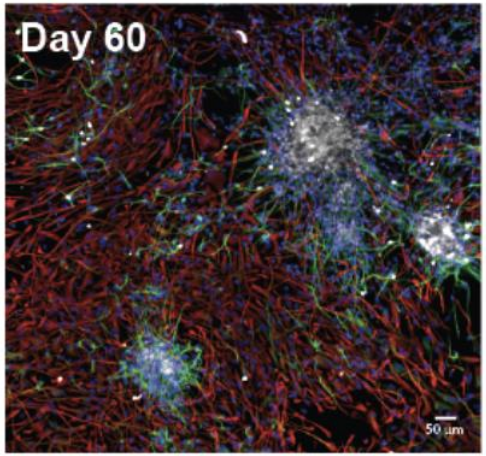




MAP2 NeuN DAPI

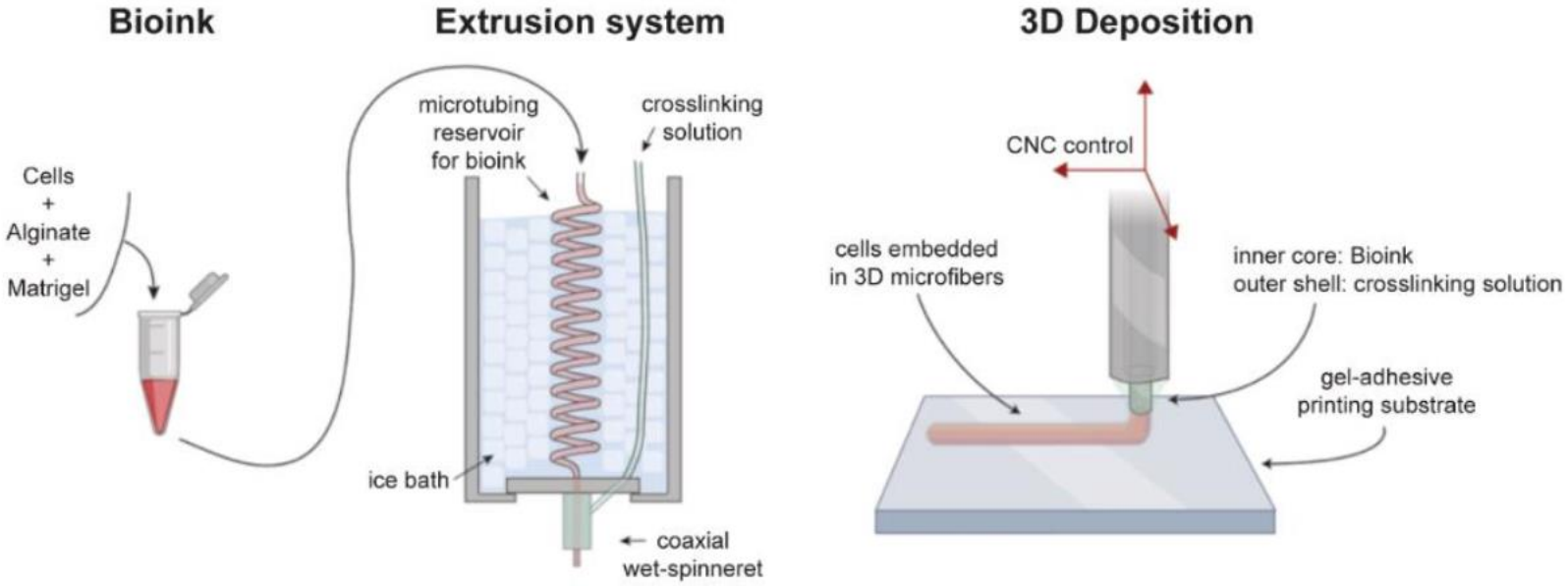


TUJ1 PAX6 DAPI



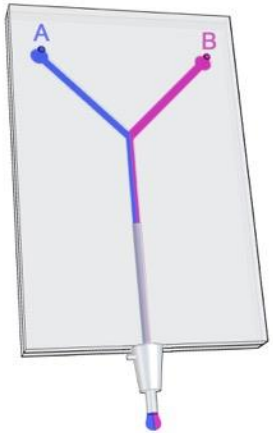
MAP2 GFAP TBR1 DAPI

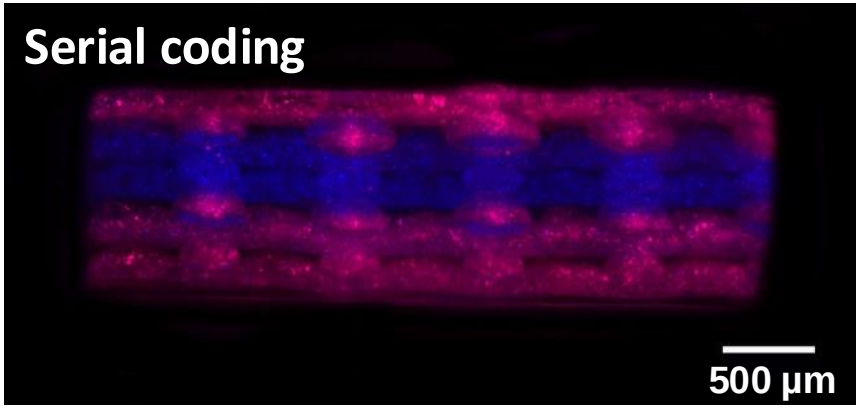
3D bioprinting of differentiating cortical neurons

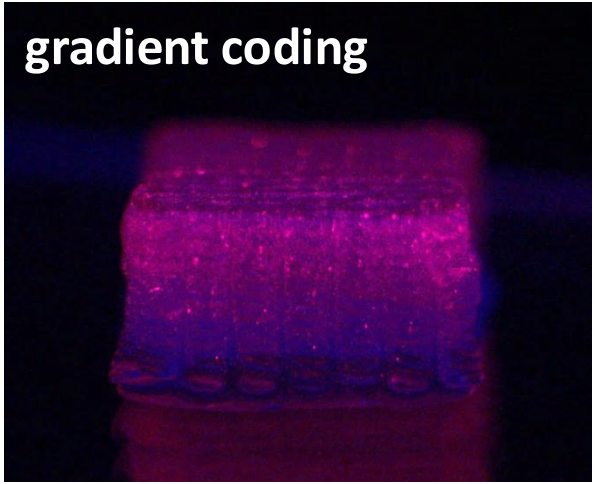


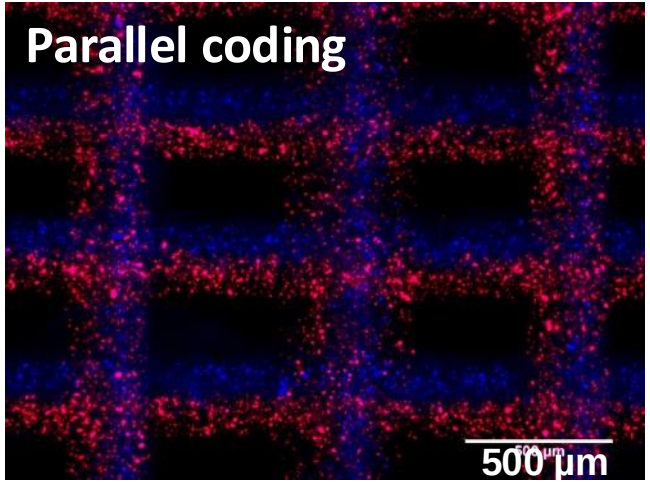
Our technology

Microfluidic print-head

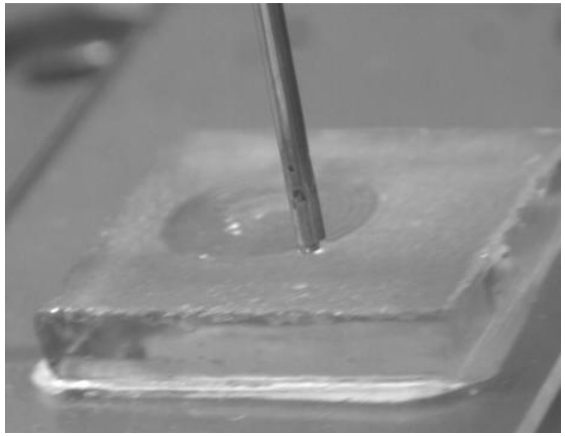


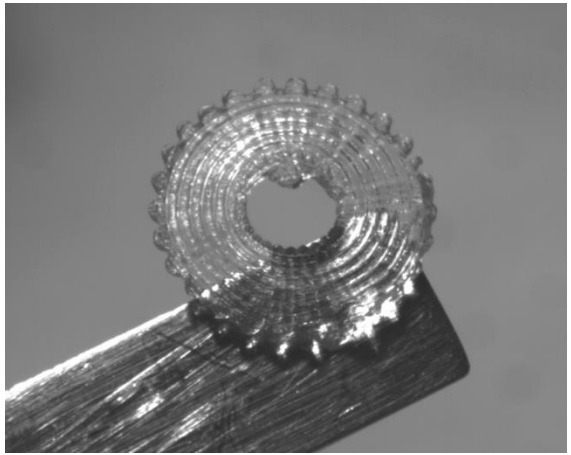






Arbitrary 3d pattern





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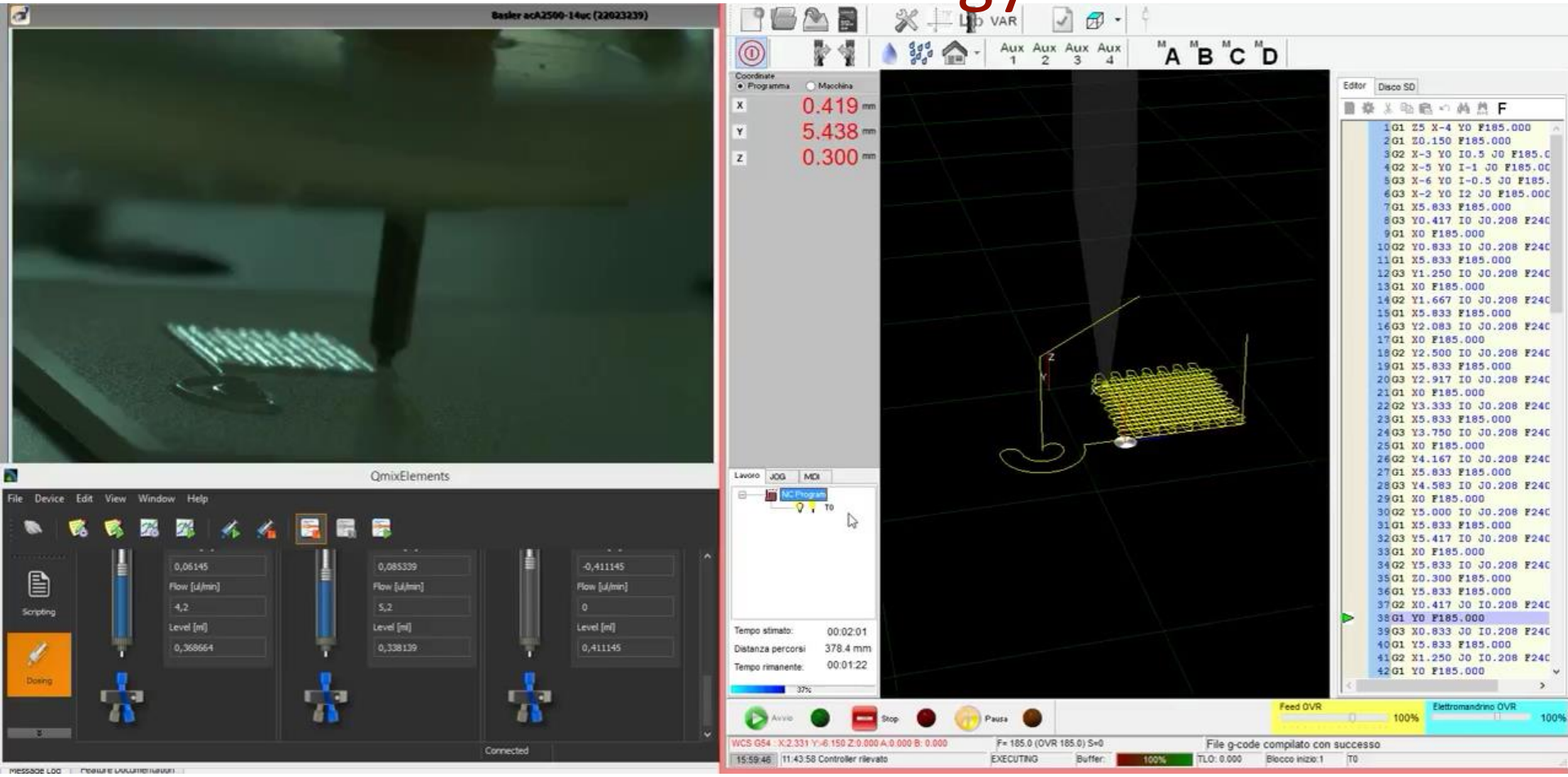
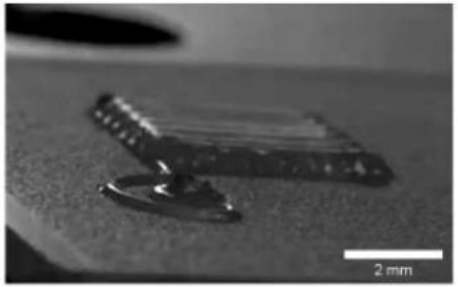
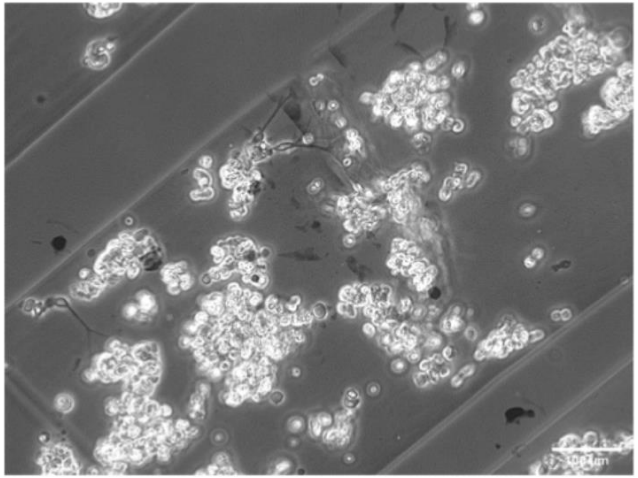
Our technology



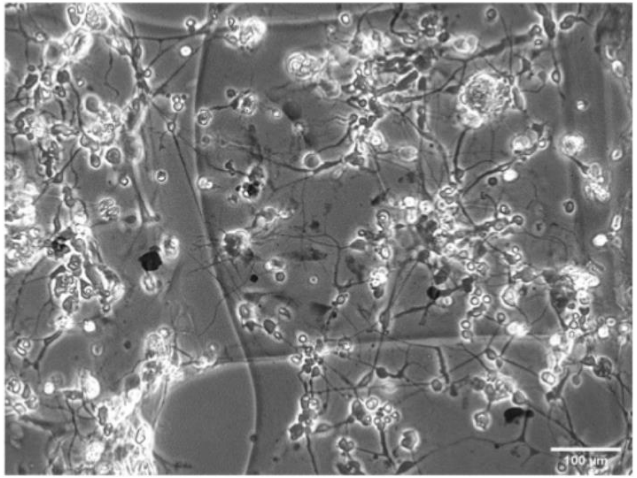
Our technology



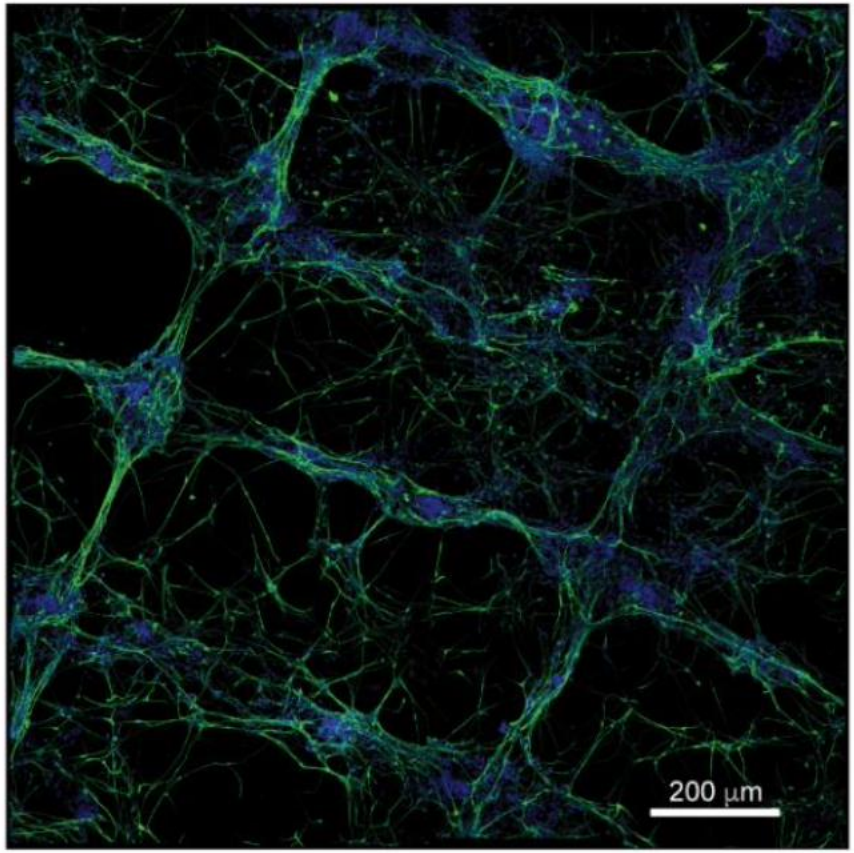
- Alginate-lyase



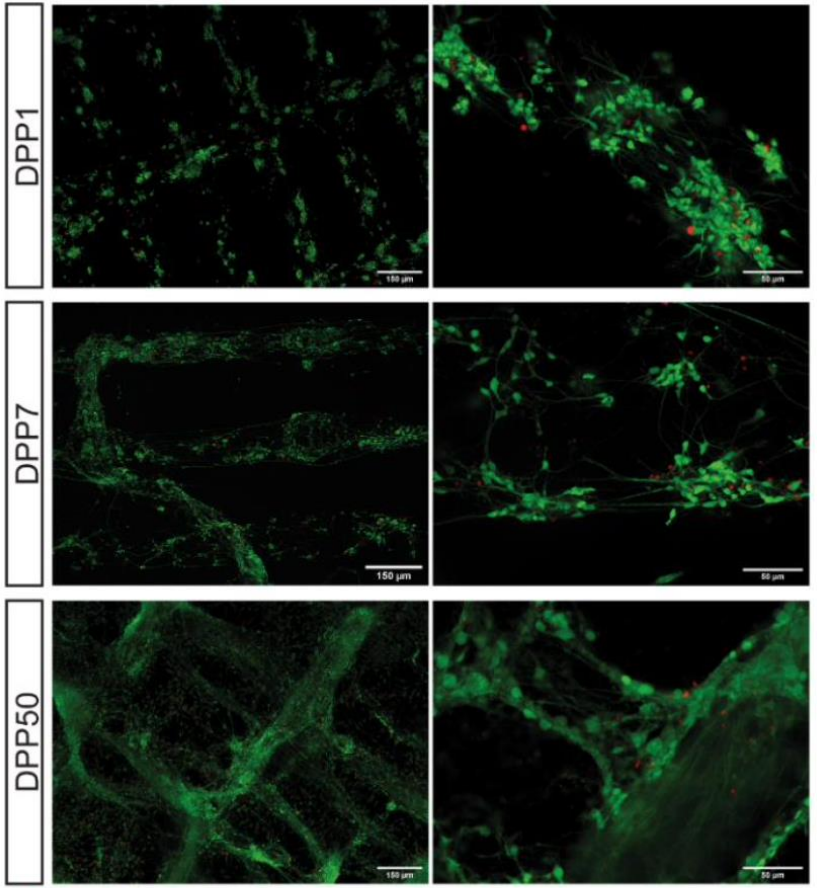
+ Alginate-lyase



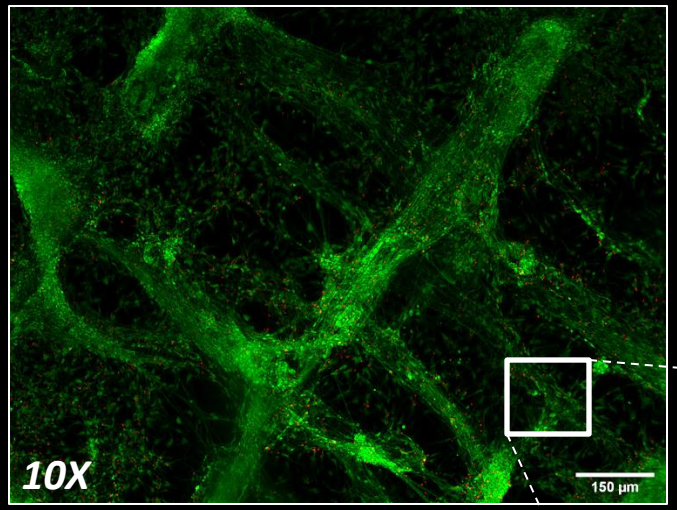
viability post printing



MAP2 DAPI

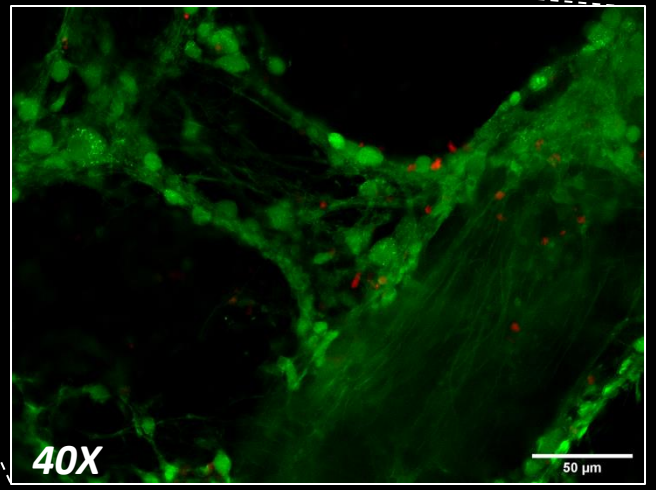


LIVE DEAD



LIVE

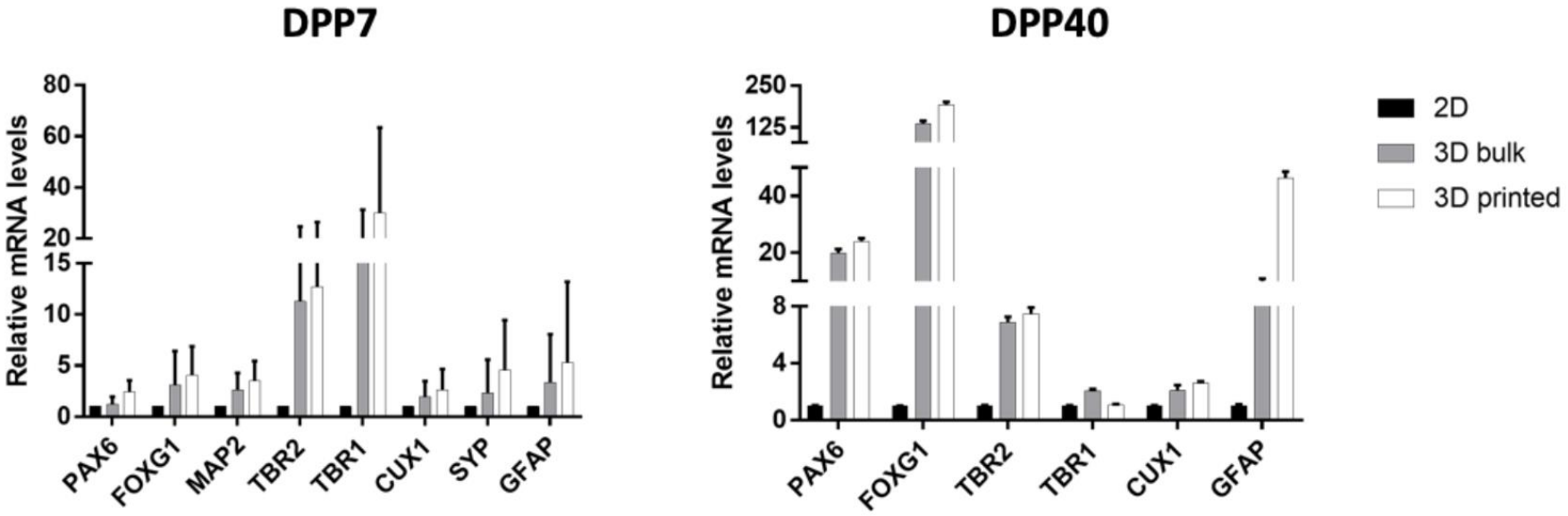
DEAD



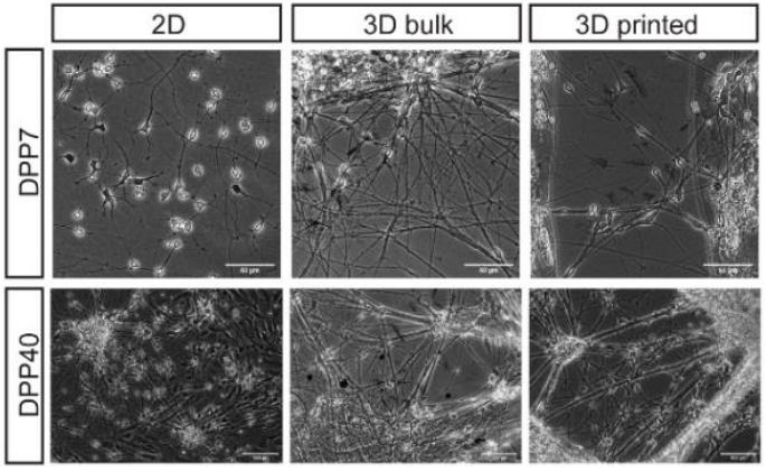
Live/dead d50 post-printing

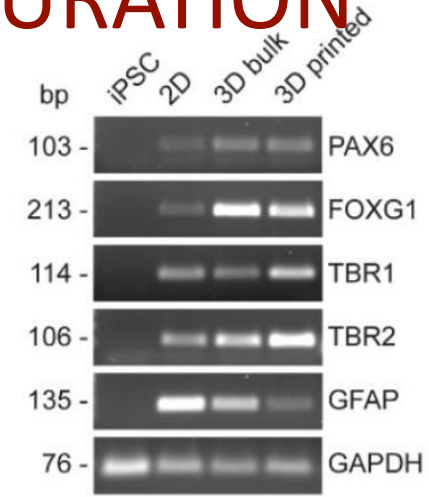
3d neural network MATURATION

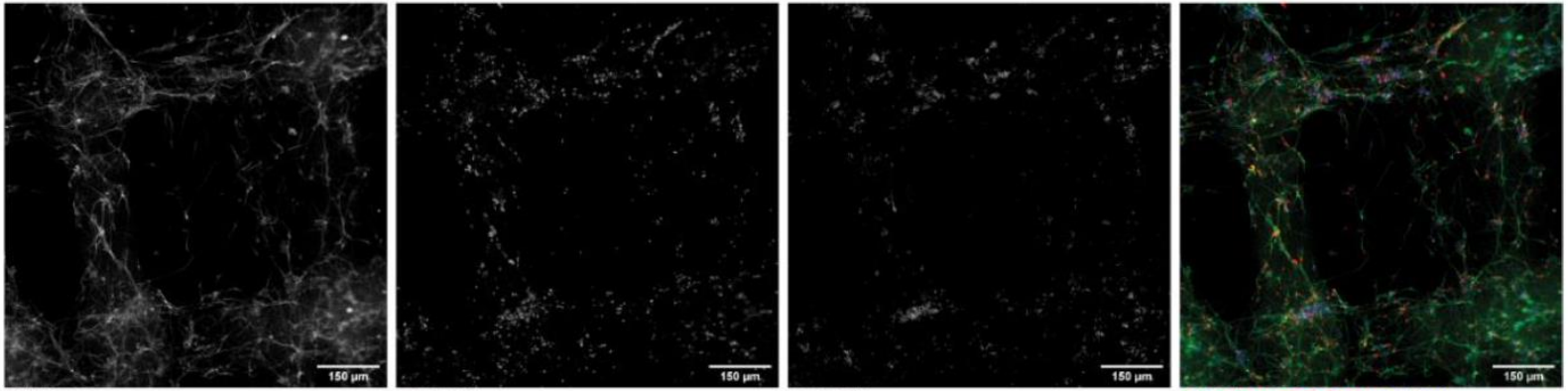
Gene expression



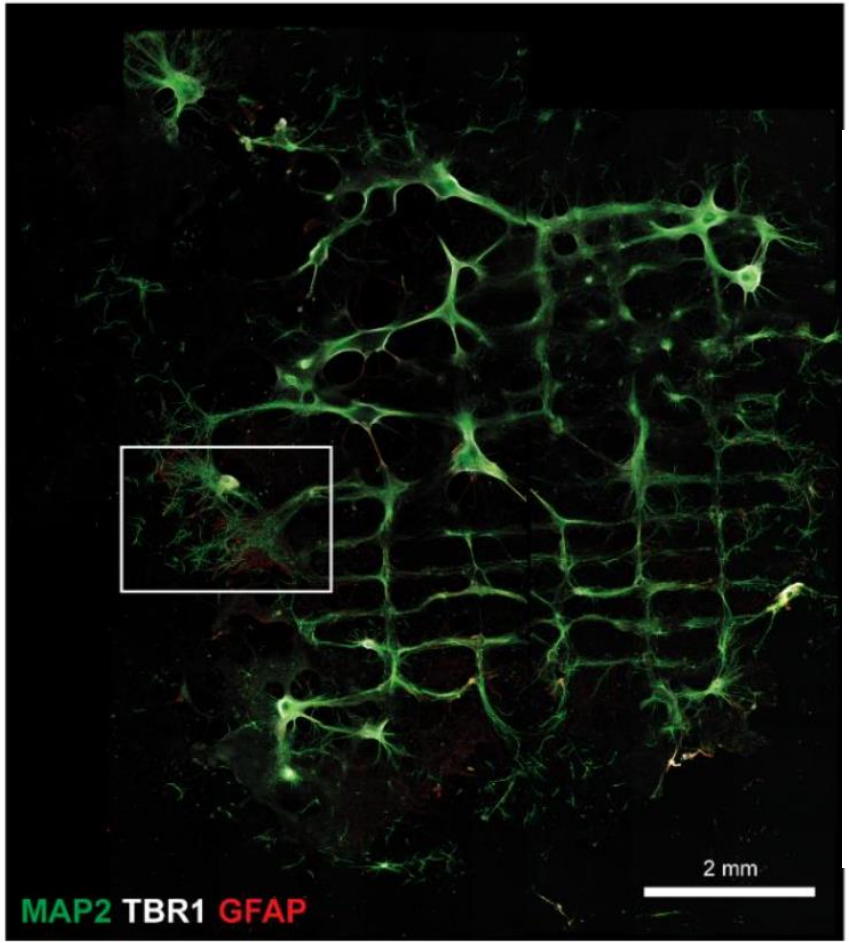
3d neural network MATURATION

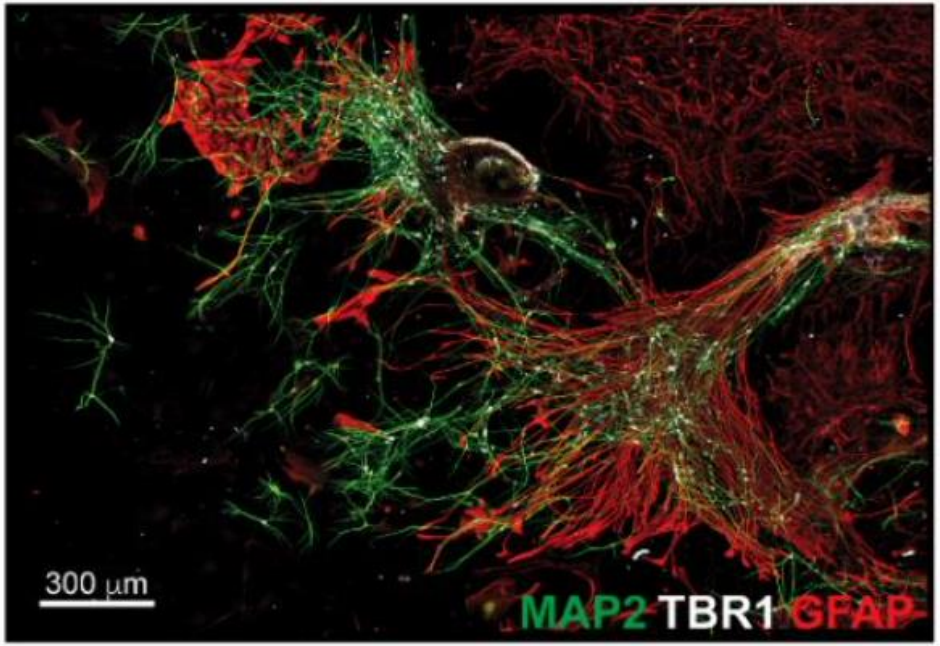






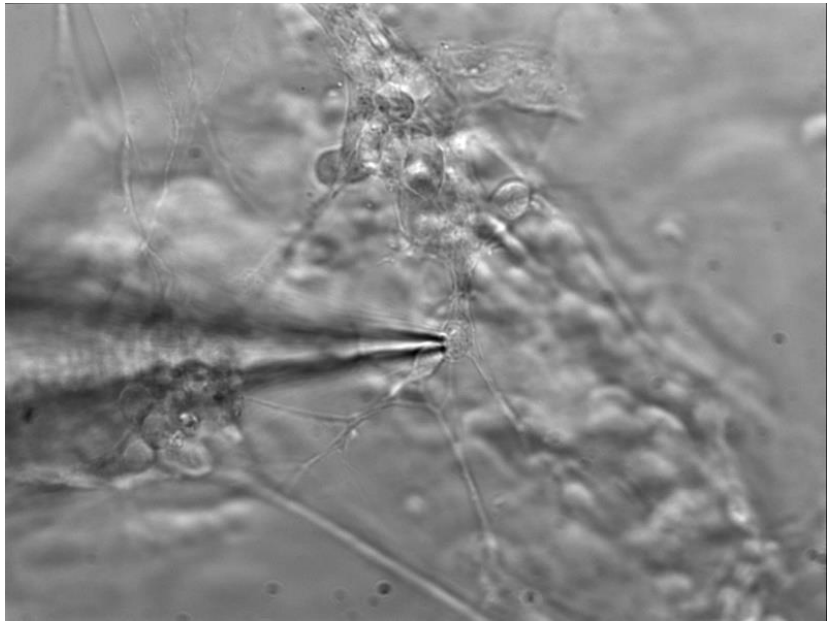
3d neural network MATURATION

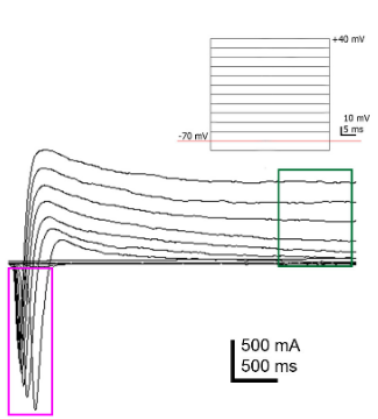


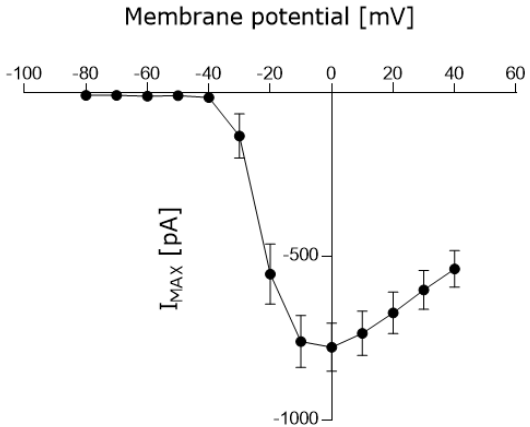


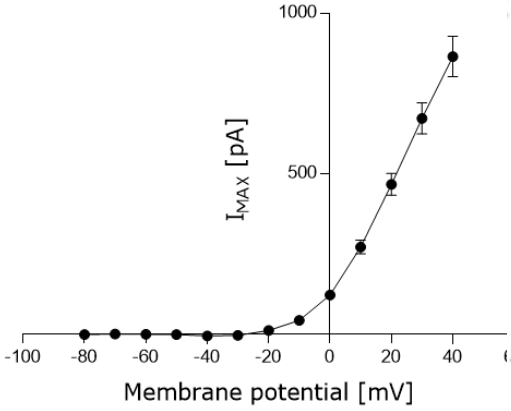
ASTROCYTES!

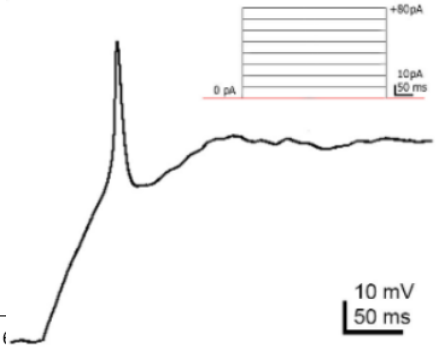
Functional ion channels in 3D printed neurons



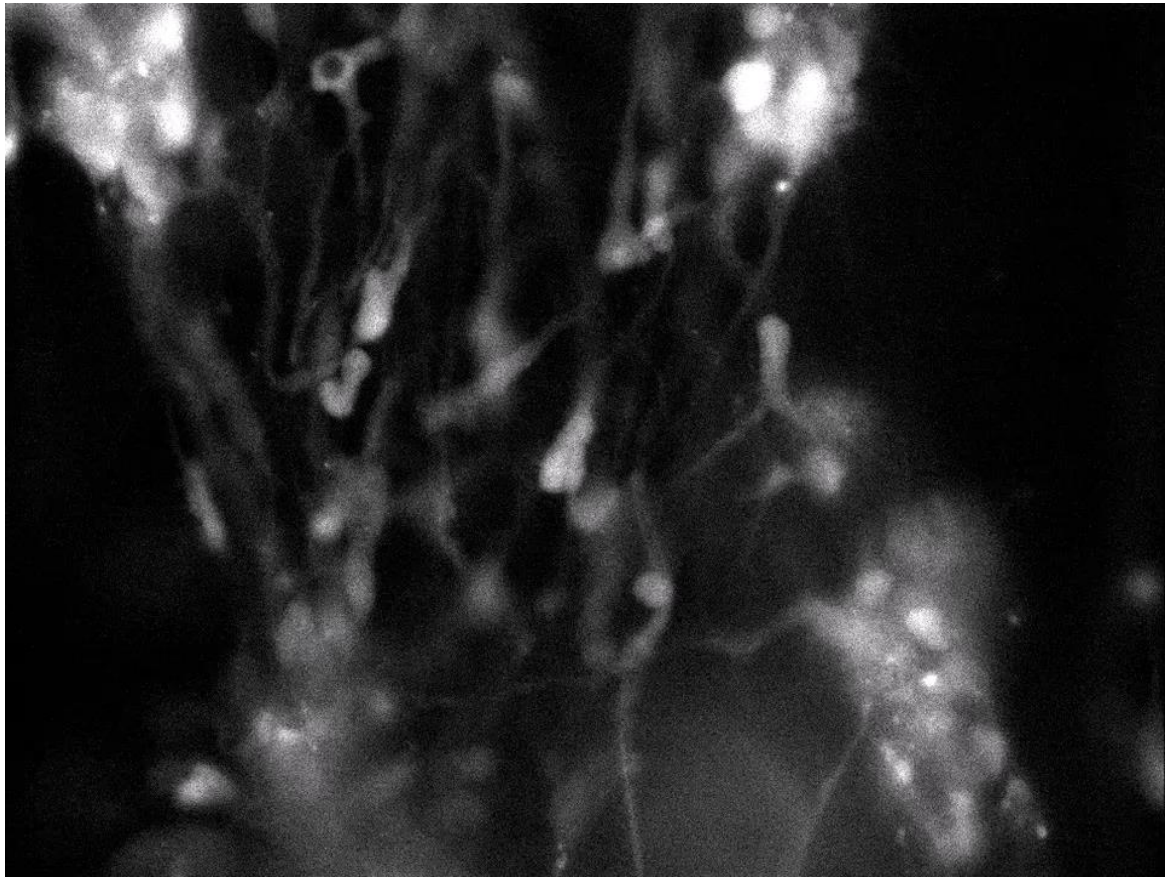


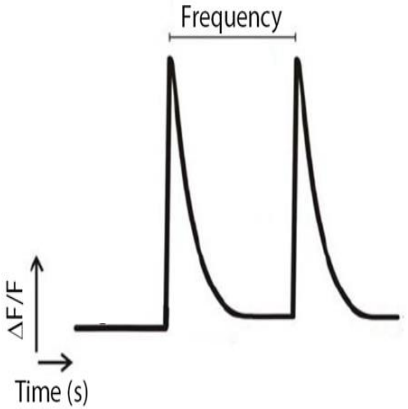




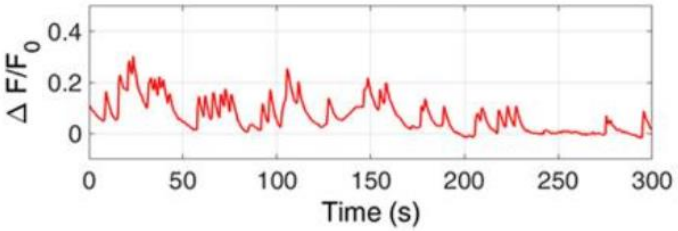


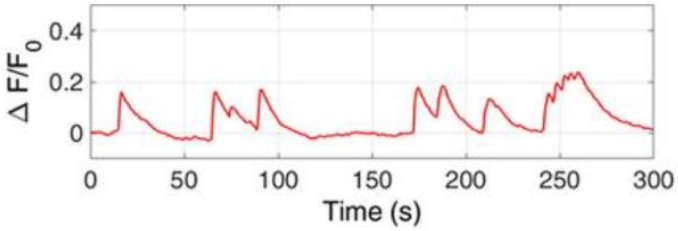
3d neural network MATURATION

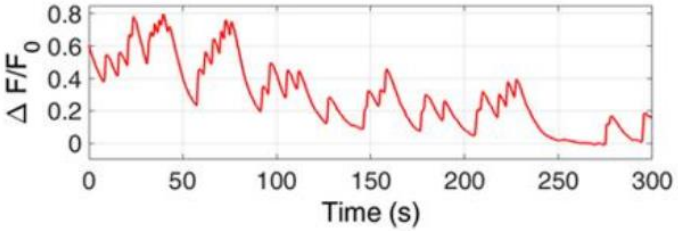


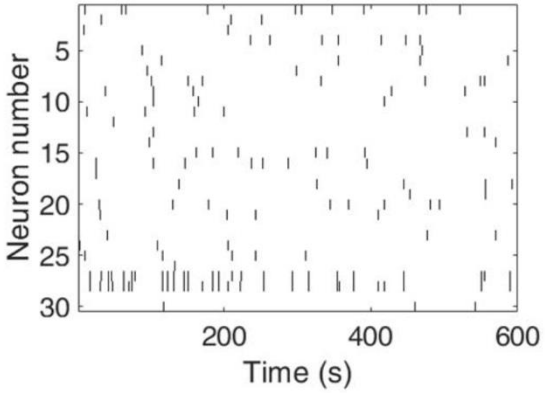


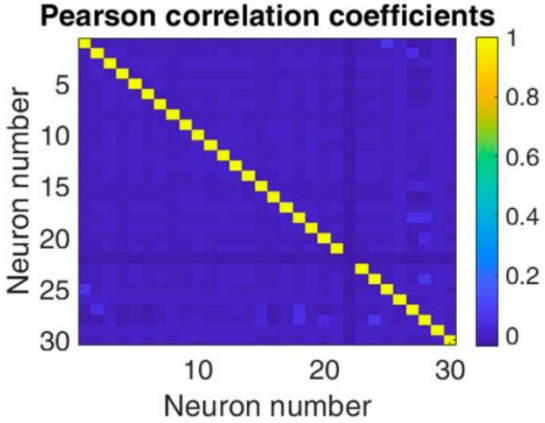
3d neural network MATURATION











ANNUAL REVIEWS

Annual Review of Neuroscience

3D Brain Organoids: Studying Brain Development and Disease Outside the Embryo

Silvia Velasco,[1,2] Bruna Paulsen,[1,2] and Paola Arlotta[1,2]

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ARTICLE

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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[1], Andrew P. Jackson[2] & Juergen A. Knoblich[1]

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Present and future of modeling human brain development in 3D organoids

Giorgia Quadrato[1,2] and Paola Arlotta[1,2]

Current Opinion in Cell Biology

ZIKA VIRUS

Zika virus impairs growth in human neurospheres and brain organoids

Patricia P. Garcez,[2,1]* Erick Correia Loiola,[1]† Rodrigo Madeiro da Costa,[1]† Luiza M. Higa,[3]† Pablo Trindade,[1]† Rodrigo Delvecchio,[3] Juliana Minardi Nascimento,[1,4] Rodrigo Brindeiro,[3] Amilcar Tanuri,[3] Stevens K. Rehen[1,2]*