Three-dimensional (3D) cell culture assays for modelling development and disease

Alexandre Bruni-Cardoso

Assistant Professor, Department of Biochemistry – Institute of Chemistry
University of São Paulo- Brazil

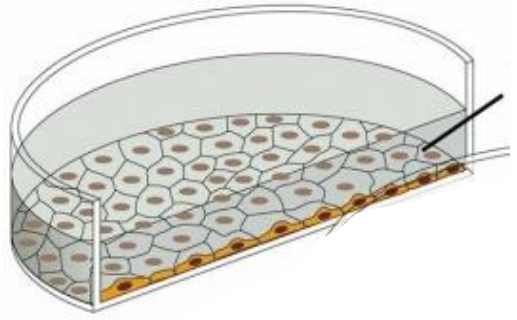
email: brunicar@iq.usp.br
homepage: www.ecm-signaling.com

What is cell culture?

"Cell culture refers to the isolation of cells from an organism and their subsequent growth in a favorable environment"

For studying:

- normal physiology and biochemistry
- effects of drugs and toxic compounds
- mutagenesis and carcinogenesis
- drug screening and development
- large scale manufacturing of biological compounds (e.g., vaccines, therapeutic proteins)



2D cell culture (monolayer)

“Goodbye flat biology”

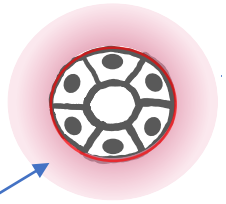
3D cell culture: An artificially-created environment in which cells are capable of interacting with their surroundings in three dimensions, and forming morphofunctional structures

Cells on 2D surface

Plastic or glass

Cells in 3D environment

vs.



Extracellular matrix

In vivo

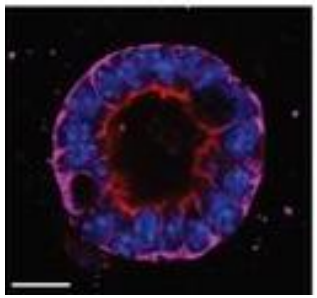
Life is 3D

Environment

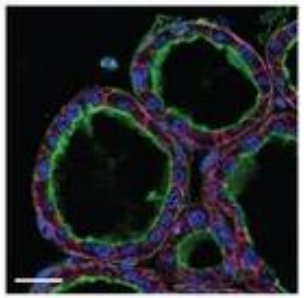
Morphology and architecture

Metabolism and response to drugs

Behavior and function



Mammary acinus in 3D culture



Mammary acini *in vivo*

2D vs 3D

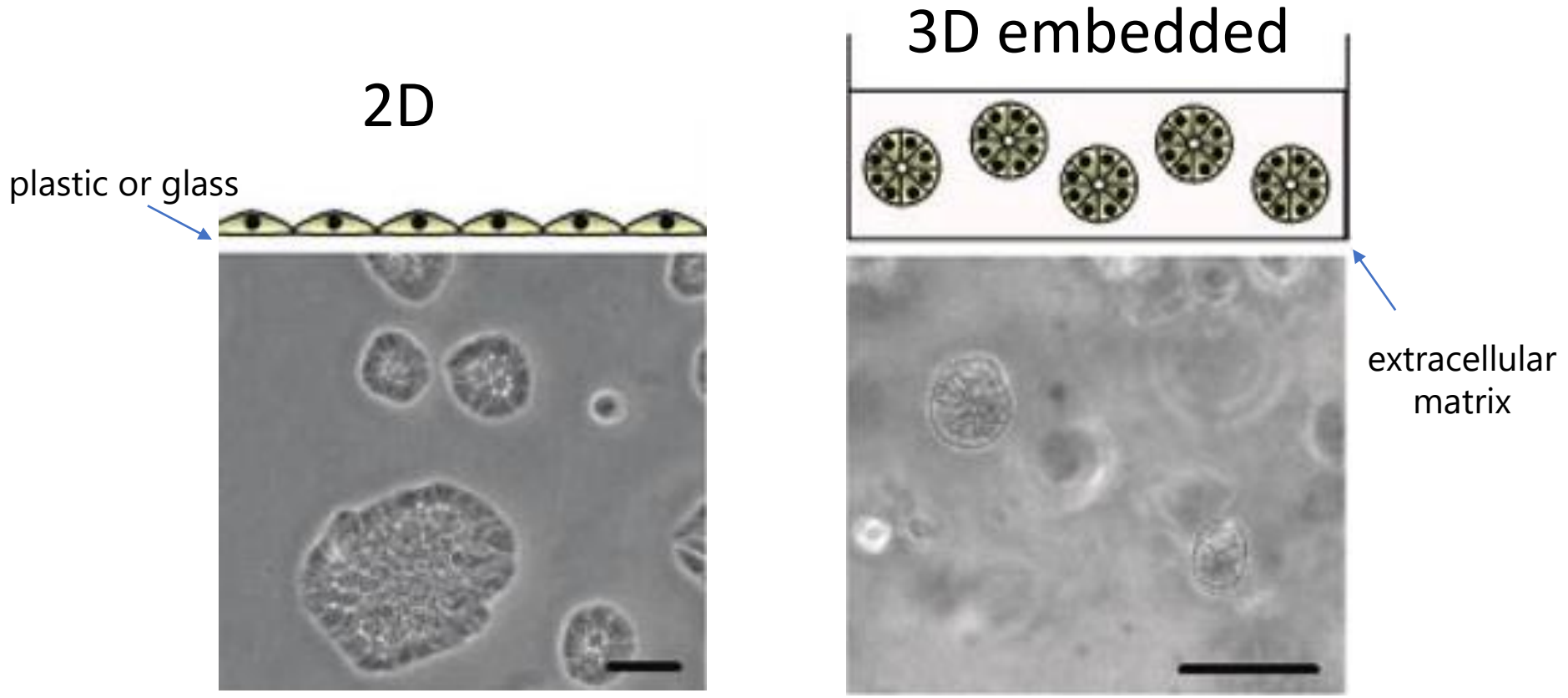


Figure modified from Lee et al, 2007 (Nat Protocols)

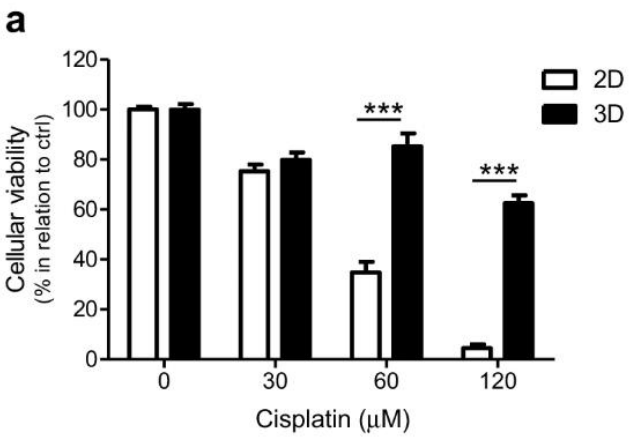
Gomes et al. *Cell Death and Disease* (2019)10:459
https://doi.org/10.1038/s41419-019-1689-8

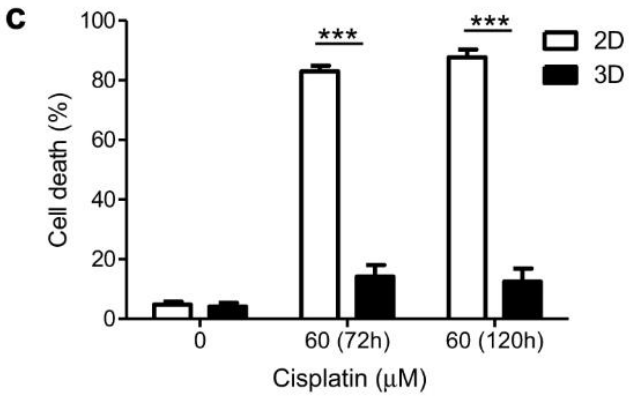
ARTICLE Open Access

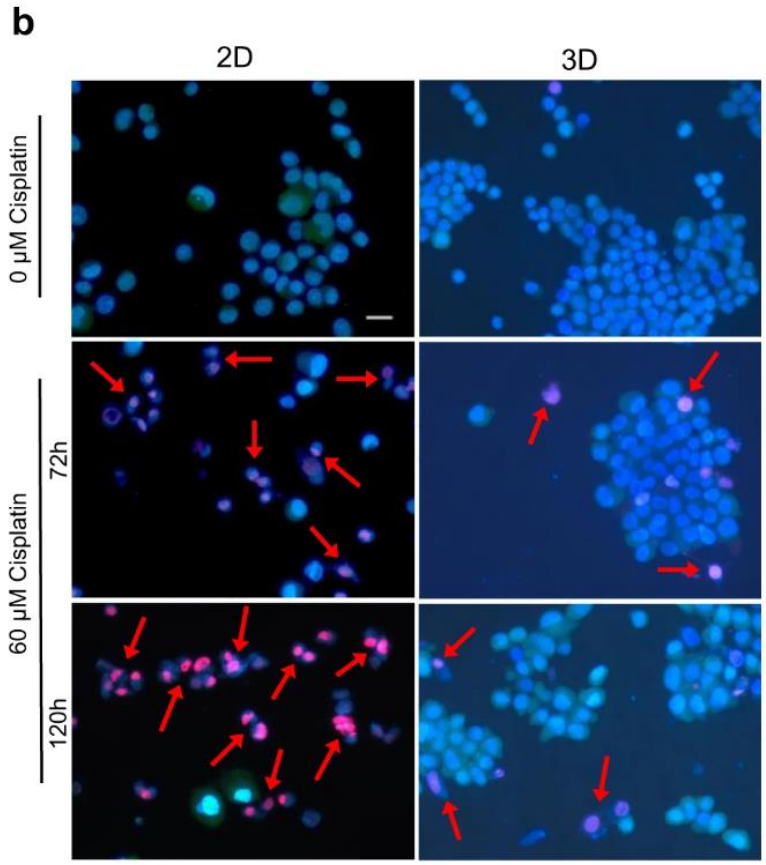
ATR mediates cisplatin resistance in 3D-cultured breast cancer cells via translesion DNA synthesis modulation

Luciana Rodrigues Gomes[1,4], Clarissa Ribeiro Reily Rocha[1,5], Davi Jardim Martins[1], Ana Paula Zen Petisco Fiore[2], Gabriela Sarti Kinker[3], Alexandre Bruni-Cardoso[2] and Carlos Frederico Martins Menck[1]

Response to drugs is different 2D vs 3D



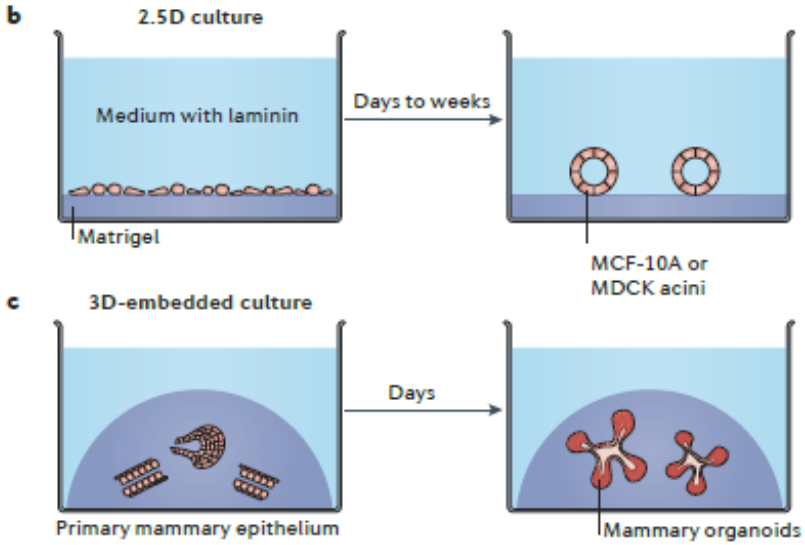


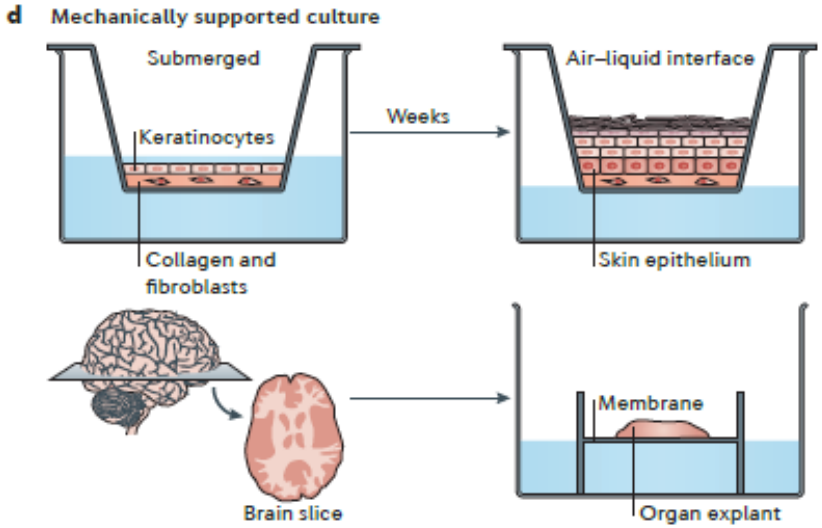


Different systems of 3D cell culture

3D cell culture can be done from:

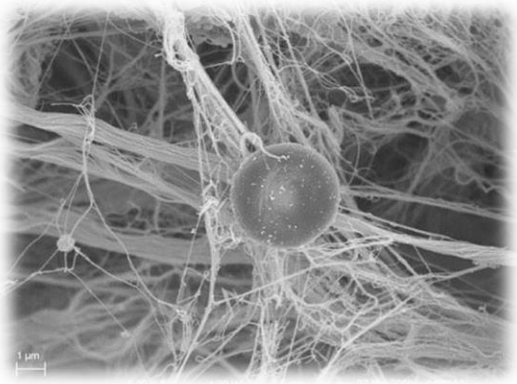
- Primary cells
- Immortalized cells
- Stem Cells (enriched, ESC and iPSC)
- Organ slices or whole organs
- On or in different substrates, usually ECM

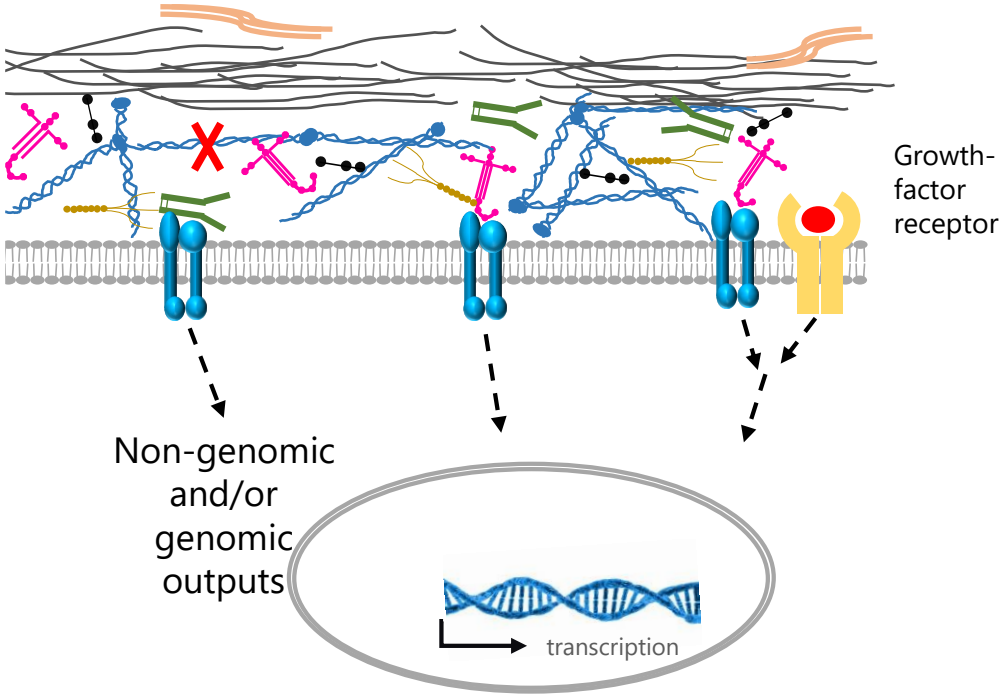


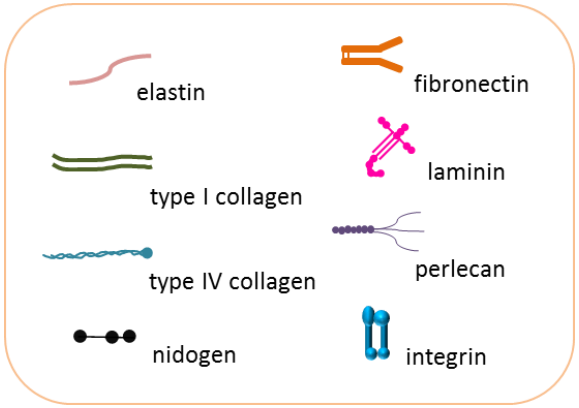


Shamir and Ewald, 2014, Nature Reviews – Molecular Cell Biology

The Extracellular Matrix (ECM) is necessary for 3D assays



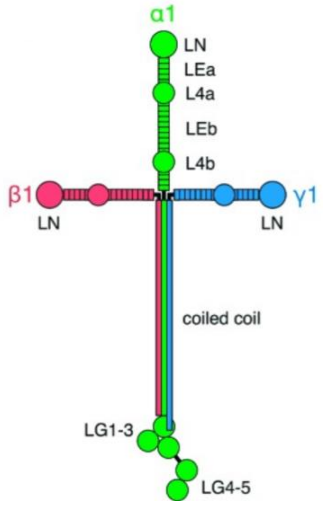




Most 3D cultures are done in a laminin-rich ECM gel (Matrigel)

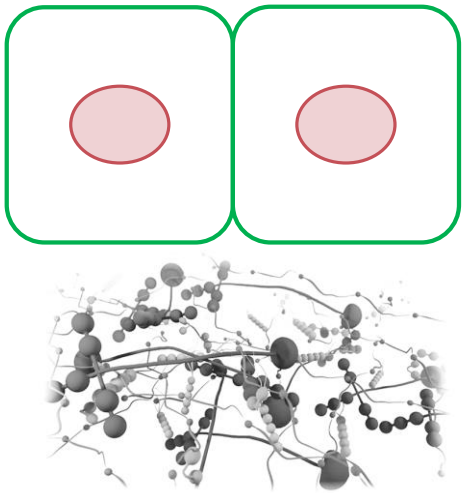
Purified from Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells

Composition:
60% Laminin,
30% collagen IV,
8% entactin
Perlecan (heparan sulfate proteoglycan)



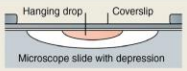
laminin

Hohenester e Yurchenco, 2013, Cell Adh Migr

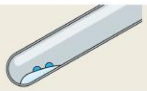


Historical timeline of 3D cell culture

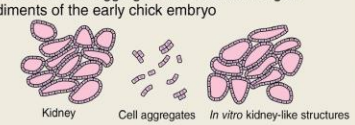
1906 Harrison
Hanging drop cell culture method



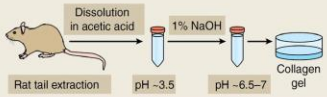
1926 Strangeways & Fell
Tube cultures



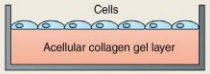
1952 Moscona & Moscona
Dissociation and aggregation of cells from organ rudiments of the early chick embryo



1956 Ehrmann & Gey
Reconstituted collagen from rat tails

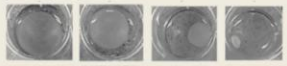


1960-1977
Culture of different cell types on collagen I gels

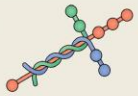


1975 Michalopoulos and Pitot
Differentiation of hepatocytes on floating collagen gels

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



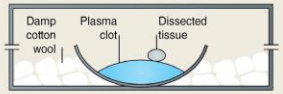
1979 Timpl et al.
Characterization of laminin



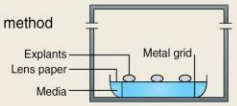
1906
1914
1926
1929
1952
1954
1956
1957
1960
1973
1975
1977
1979
1984

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

1929 Fell & Robison
Watch glass method

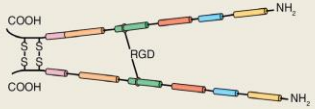


1954 Trowell
Grid cell culture method



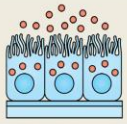
1957 Lasfargues
Dissociation of mammary glands into organoids using collagenase from *Clostridium*

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



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

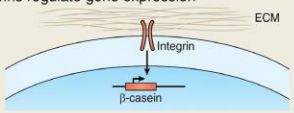
1984, 1985 Lee et al.
Basal and luminal polarity is established when cells are on floating collagen gels,as shown by milk protein synthesis and secretion into the medium.



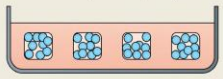
1987
1989
1991
2001
2006
2008
2009
2012
2013

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.

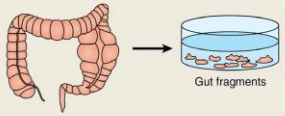
1991 Streuli et al.
Integrins regulate gene expression



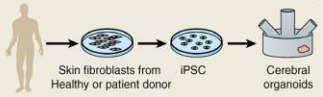
2006 Nelson et al.
Micropattern gels provide positional cues that establish the range of action of TGF-β in morphogenesis vs invasion



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

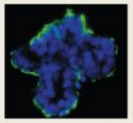


2013 Lancaster et al.
Human brain organoids are generated from iPSCs derived from cells from a patient with microcephaly.

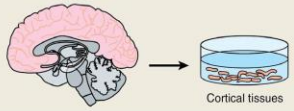


1989 Barcellos-Hoff et al. 1992 Petersen et al.
Use of a laminin-rich matrix to develop assays of mammary morphogenesis and to distinguish between healthy and malignant human epithelial cells.

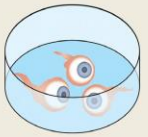
2001 Simian et al.
Use of 3D collagen cultures to study the mechanisms of mammary gland branching morphogenesis



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

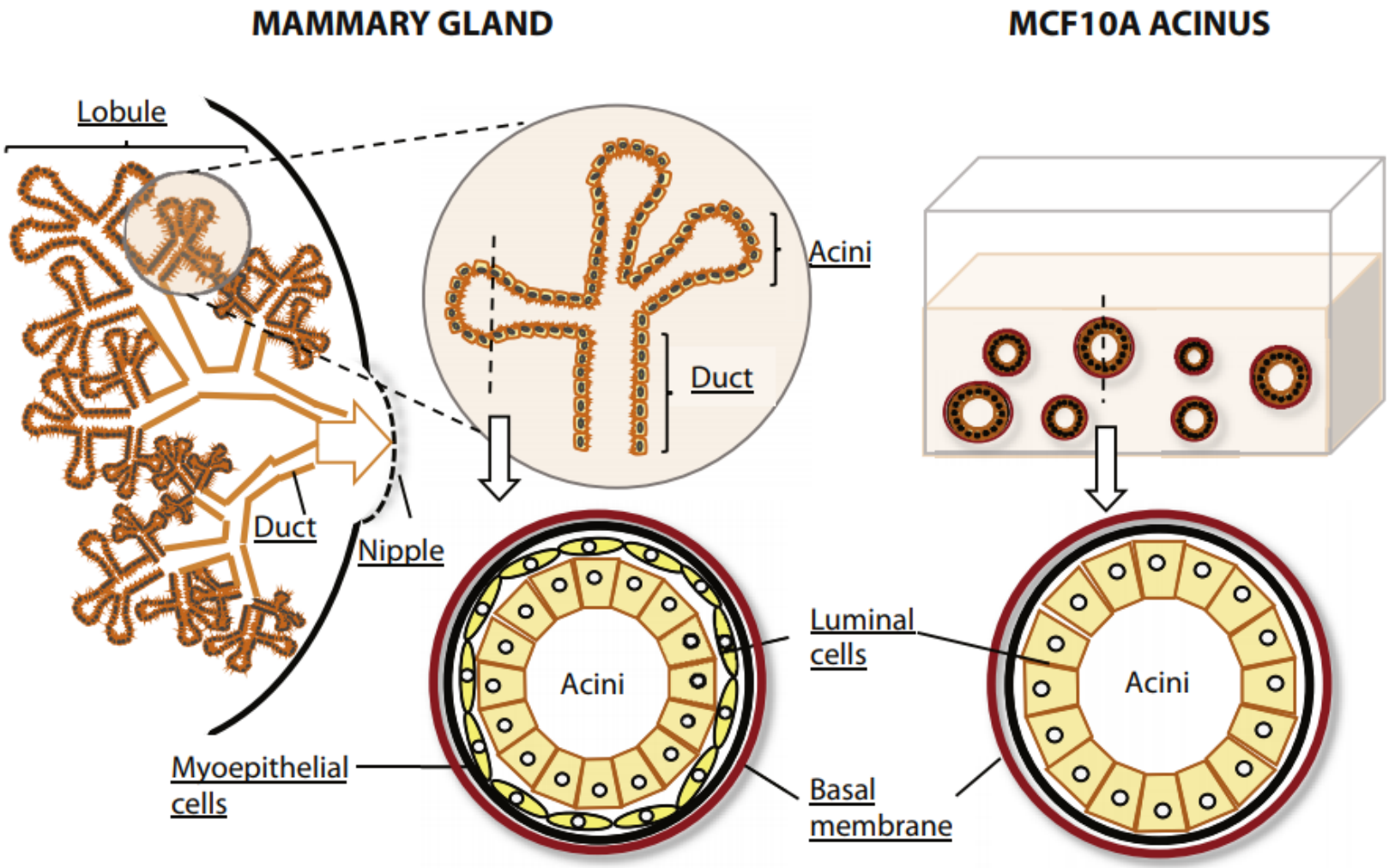


2012 Nakano et al.
Formation of a self-organized optic cup structure from human ESCs in 3D culture



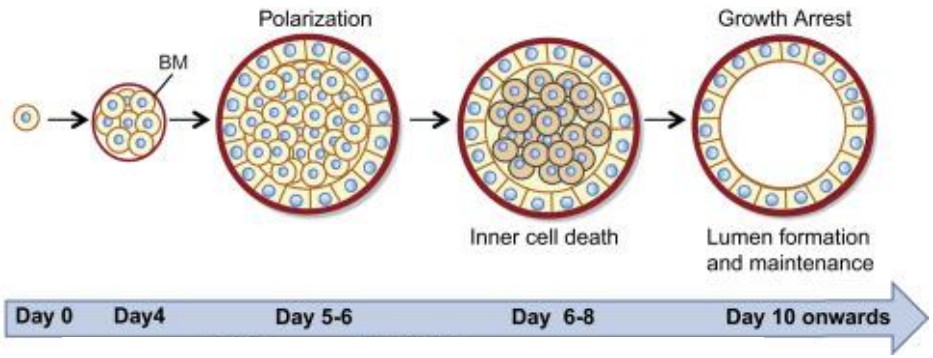
Simian and Bissell JCB, 2016

3D epithelial acinogenesis assay

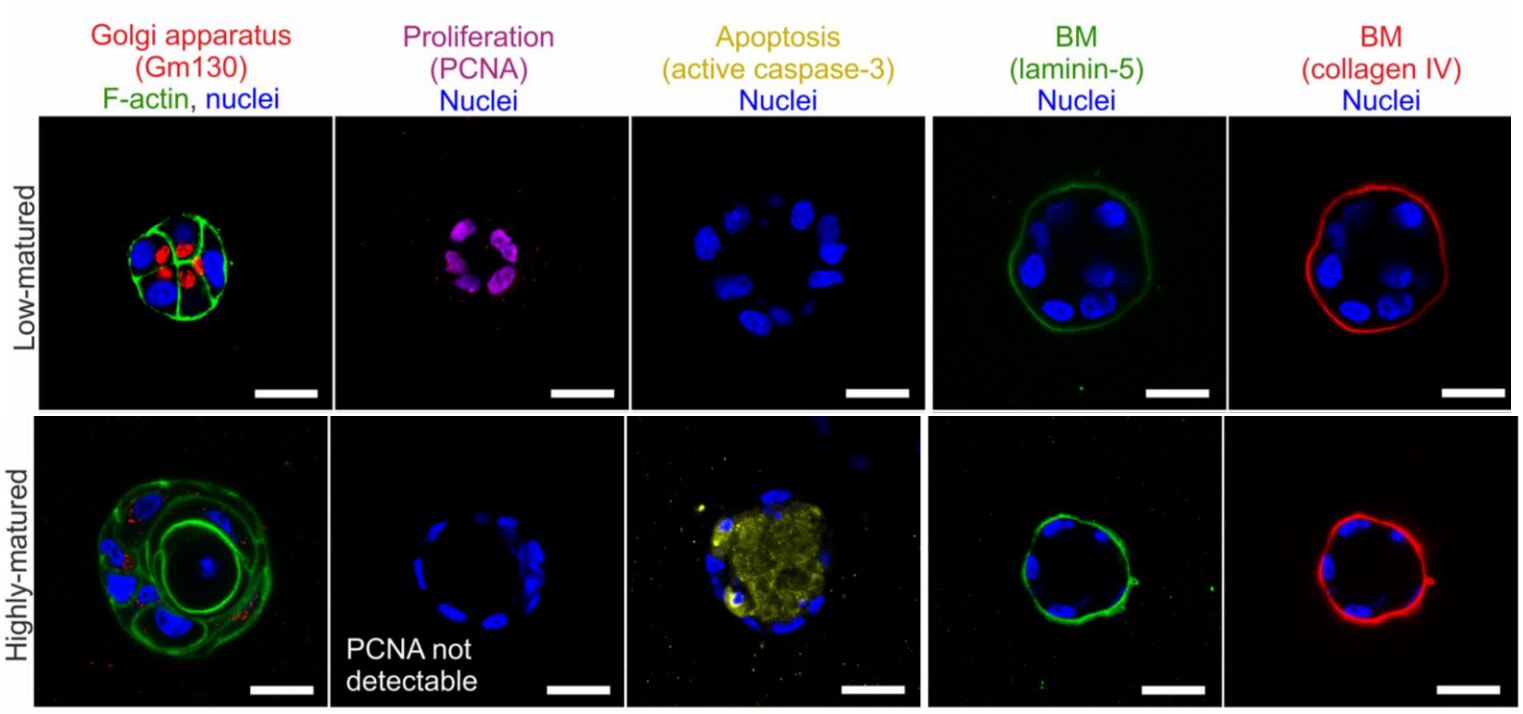


Arnandis and Godinho, 2015

3D epithelial acinogenesis assay



Arnandis and Godinho, 2015

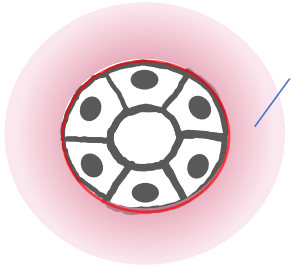


Gaiko-Shcherbak et al., 2015 Plos One

A 3D-cell culture model for functional differentiation of the mammary gland

Cells on 2D: monolayer, undifferentiated and proliferative

Basal culture medium + **lactogenic hormones**

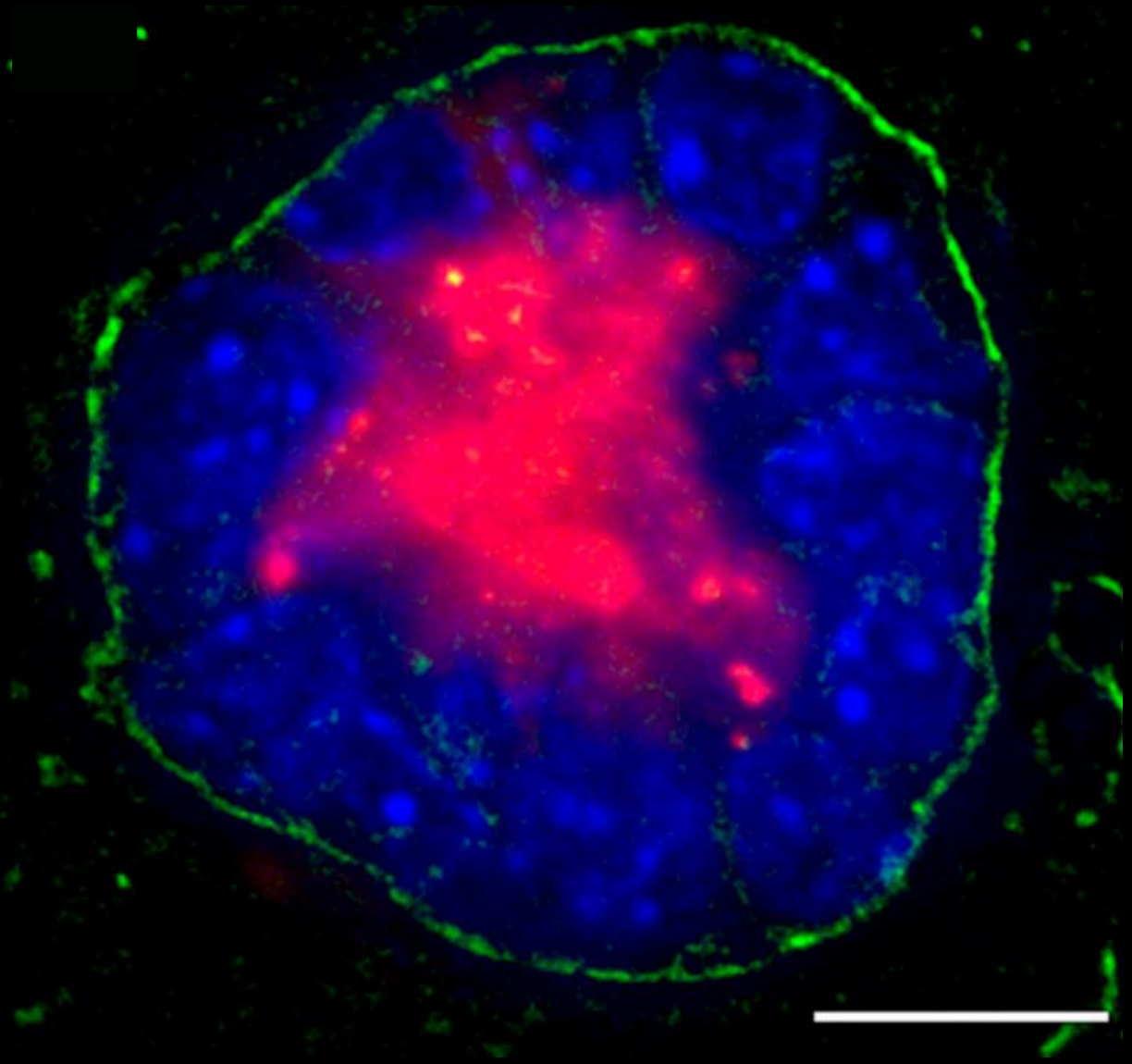


Cells in 3D: polarized, quiescent and differentiated→ synthesis of lactoferrin, β-casein and whey acidic protein

Basal Culture medium + laminin-rich gel + lactogenic hormones

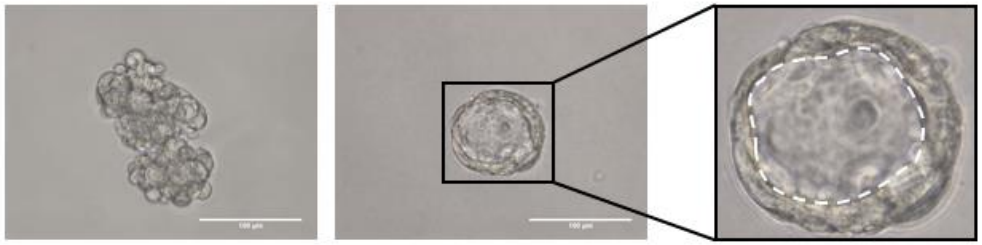
Schematic based on: Barcellos-Hoff et al. 1989, JCS; Ren, Streuli et al. 1991 e 1995, JCB; Muschler et al. 1999, Mol Biol Cell;

Nucleus basement membrane β-casein

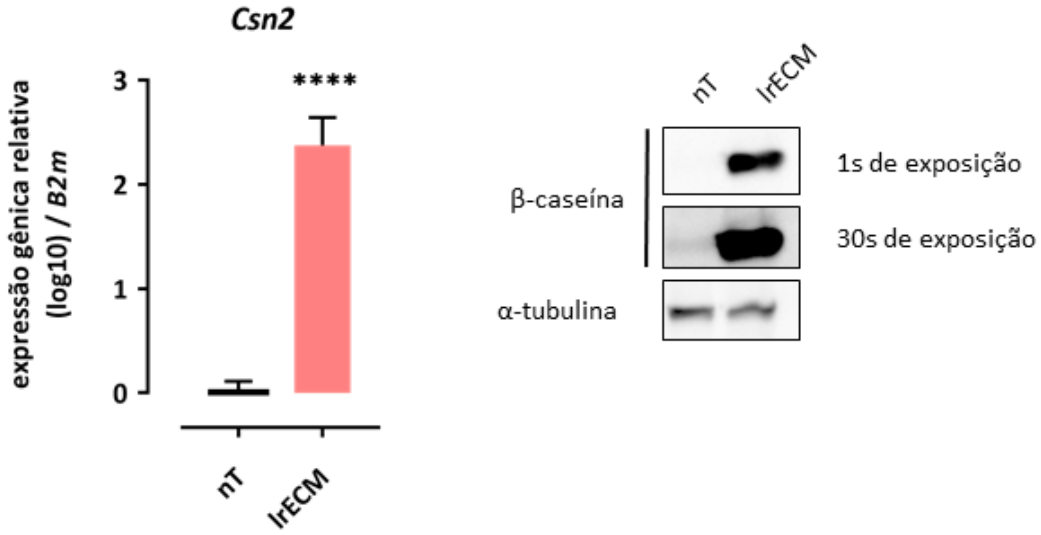


3D-mammary acinus – confocal microscopy (Streuli lab)

A laminin-rich ECM is necessary for beta-casein expression

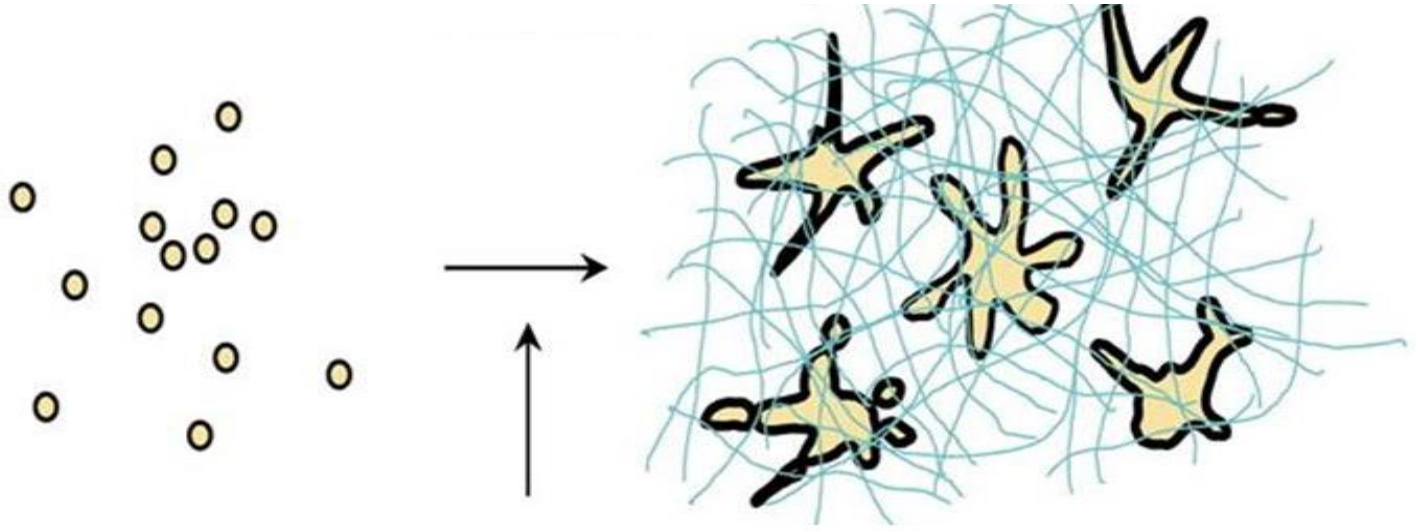



Antonio Manucci, PhD candidate



Antonio Manucci et al, in preparation

“Branching assay” : a 3D assay that reproduces epithelial branching and invasion

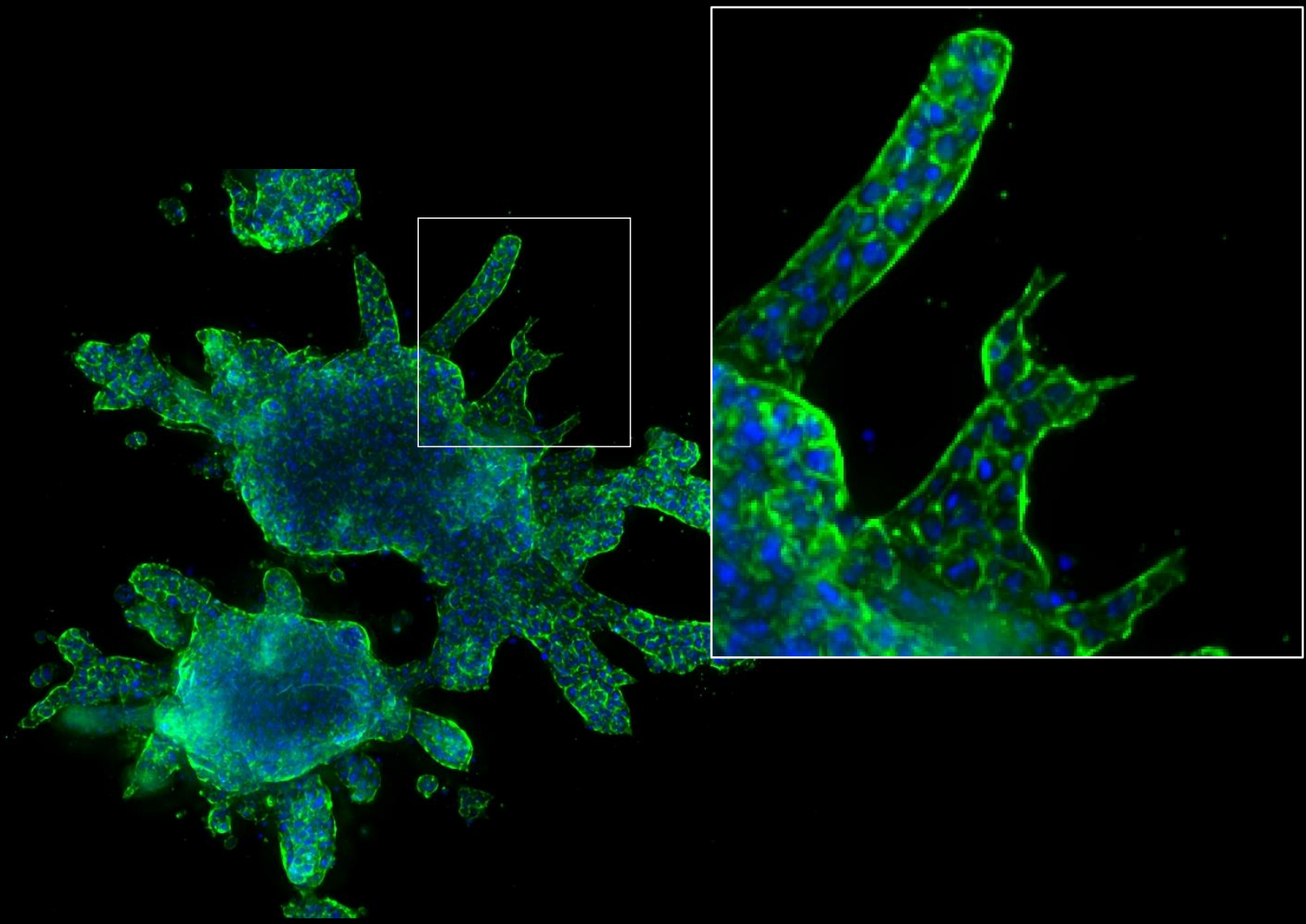


Individualized Mouse mammary epithelial cells

FGF2 or EGF

4 days in collagen type I gels

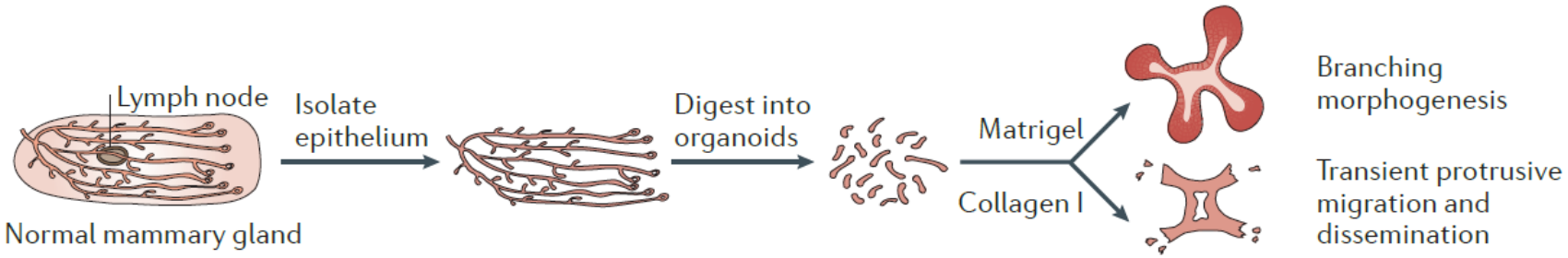
Branching/invasion assay in collagen gels



Nuclei
F-actin cytoskeleton

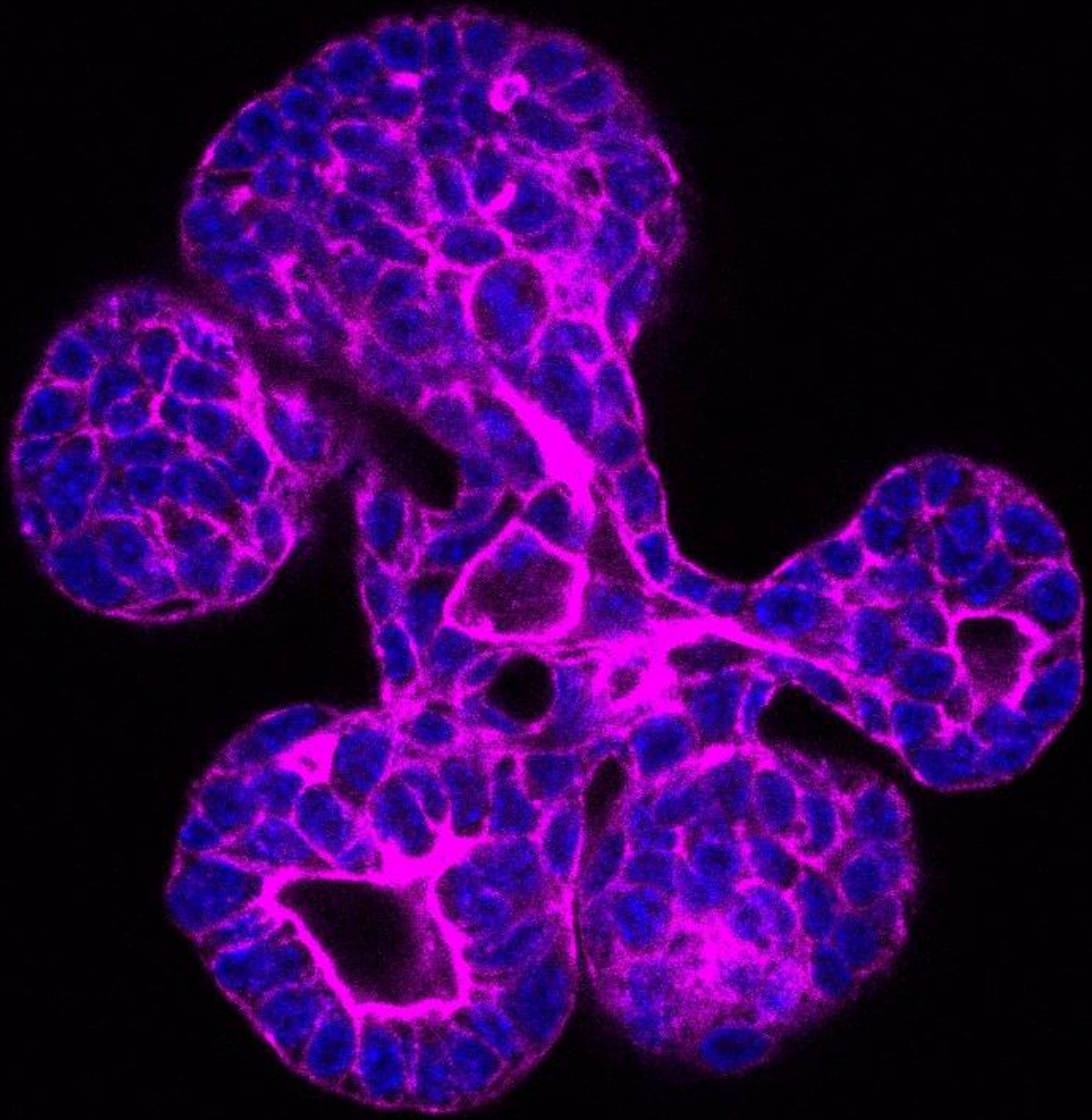
Images by Giovani Genesi (Masters student in the e-Signal lab)

Mammary organoid: a 3D assay that reproduces epithelial alveologenesis or invasion



Shamir and Ewald, 2014, Nature Reviews – Molecular Cell Biology

3D organoid



Nuclei
F-actin cytoskeleton

Bruni-Cardoso, in preparation

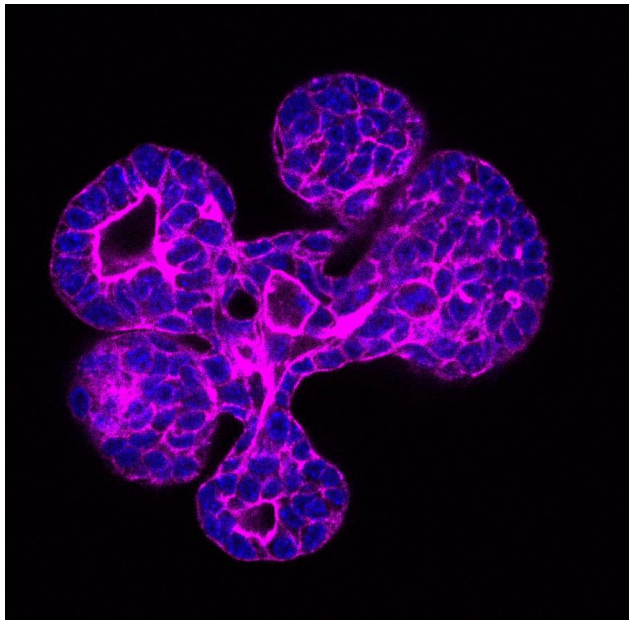
Organoids are a subtype of 3D culture

"(…) "in the field of mammary gland biology, the term organoids refers to primary explants of epithelial ducts into 3D extracellular matrix (ECM) gels.

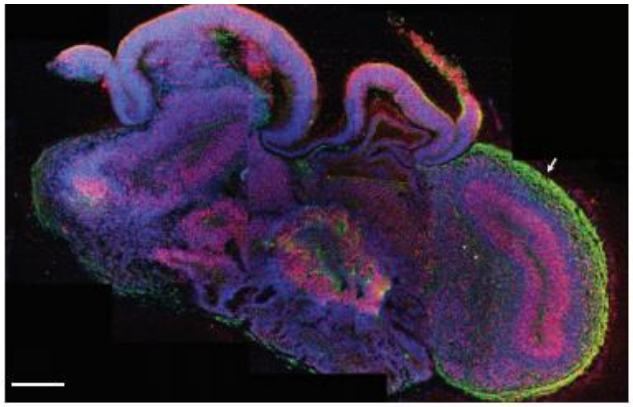
Conversely, in studies of intestinal biology, organoids can refer to clonal derivatives of primary epithelial stem cells that are grown without mesenchyme or can refer to epithelial–mesenchymal co-cultures that are derived from embryonic stem cells or induced pluripotent stem cells"

(Shamir and Ewald, 2014)

Mammary gland organoid



Cerebral organoid



Lancaster et al, 2013, Nature

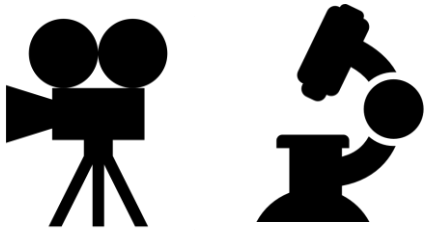
"The journey, not the destination matters“ T.S. Eliot

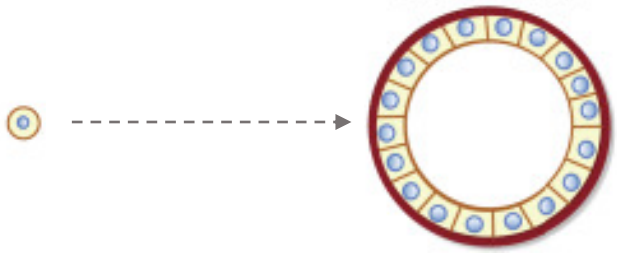
"The journey, not the destination matters“ T.S. Eliot

"The journey, not the destination matters“ T.S. Eliot

Video maker: Rebeka Tomasin

4D microscopy





single cell

acinus

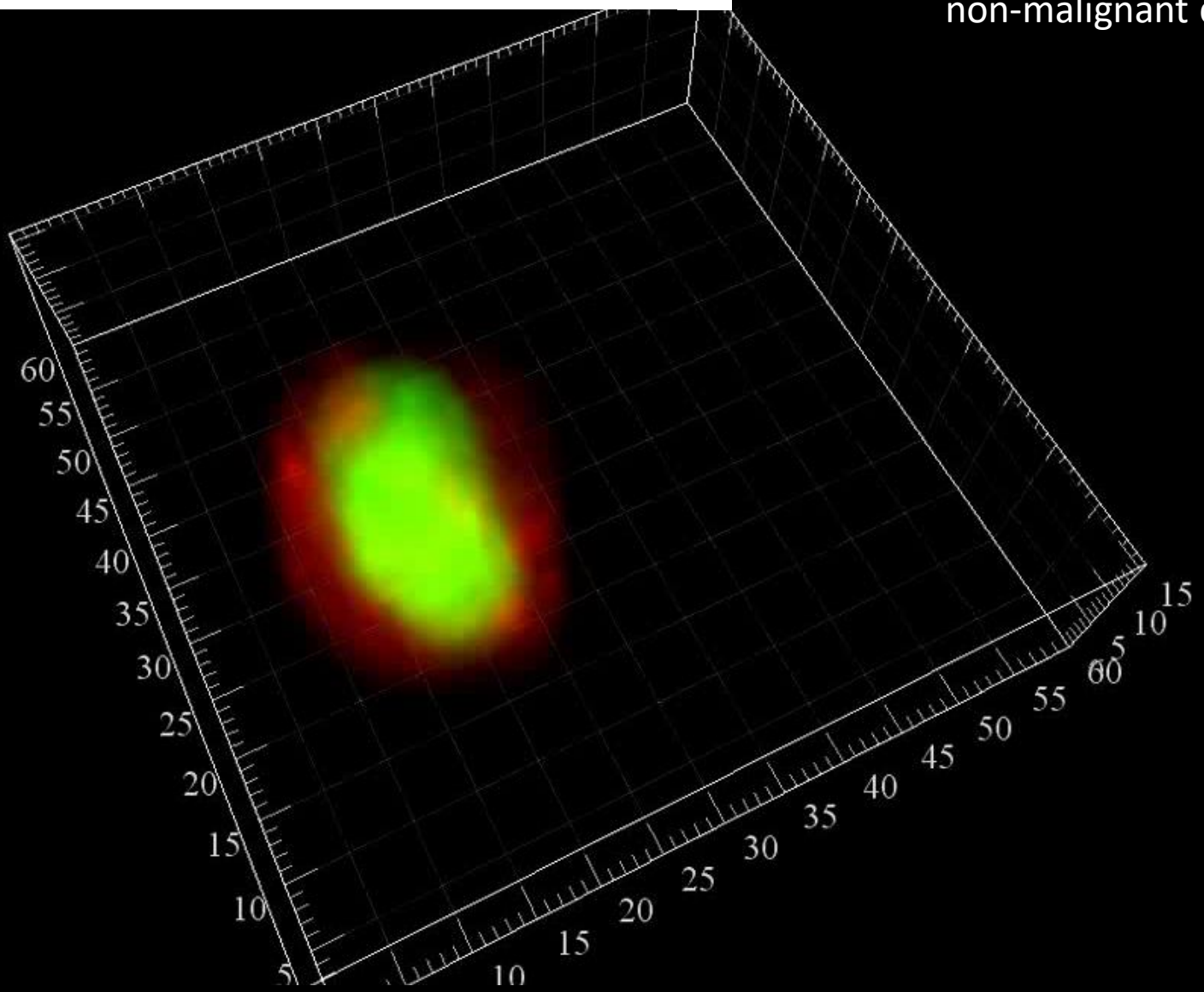
PNAS

Coherent angular motion in the establishment of multicellular architecture of glandular tissues

Kandice Tanner[1], Hidetoshi Mori, Rana Mroue, Alexandre Bruni-Cardoso, and Mina J. Bissell[1]

Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

non-malignant cells



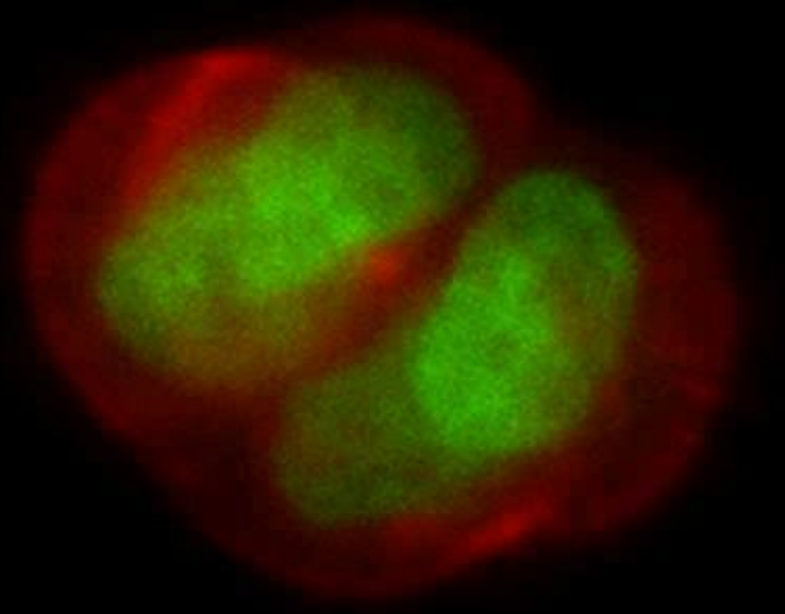
PNAS

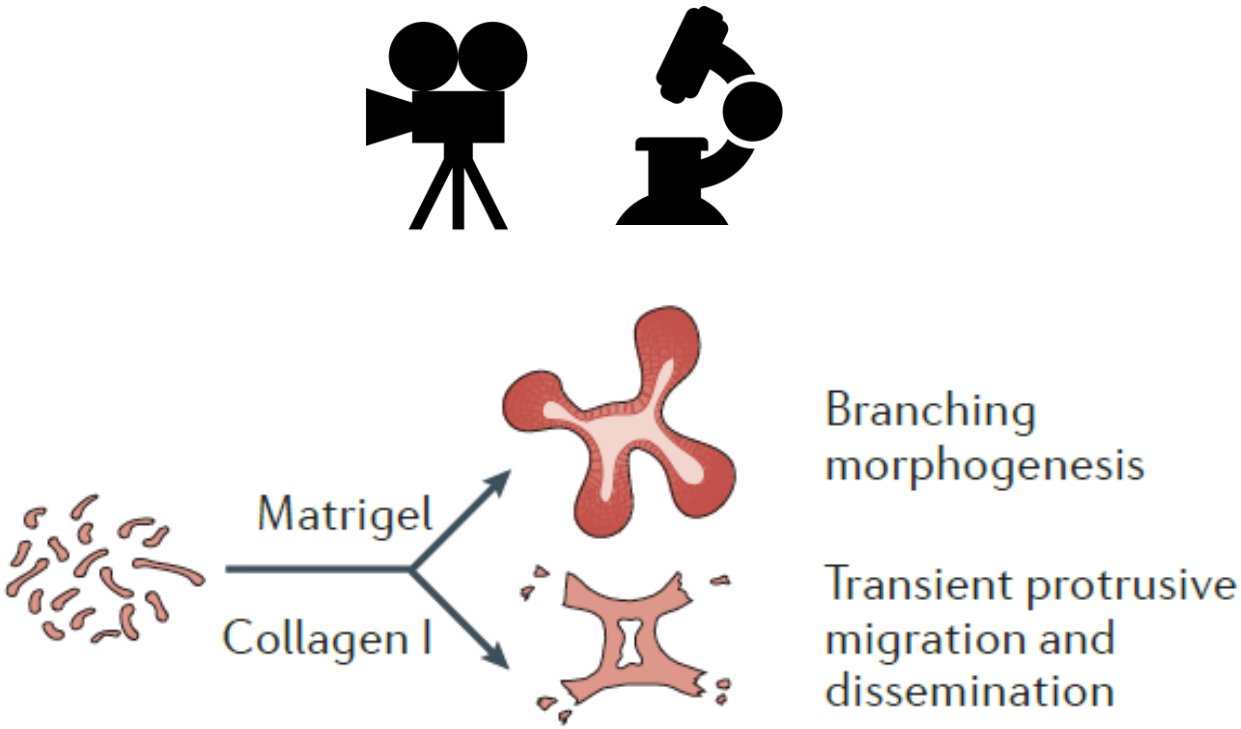
Coherent angular motion in the establishment of multicellular architecture of glandular tissues

Kandice Tanner[1], Hidetoshi Mori, Rana Mroue, Alexandre Bruni-Cardoso, and Mina J. Bissell[1]

Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

malignant cells



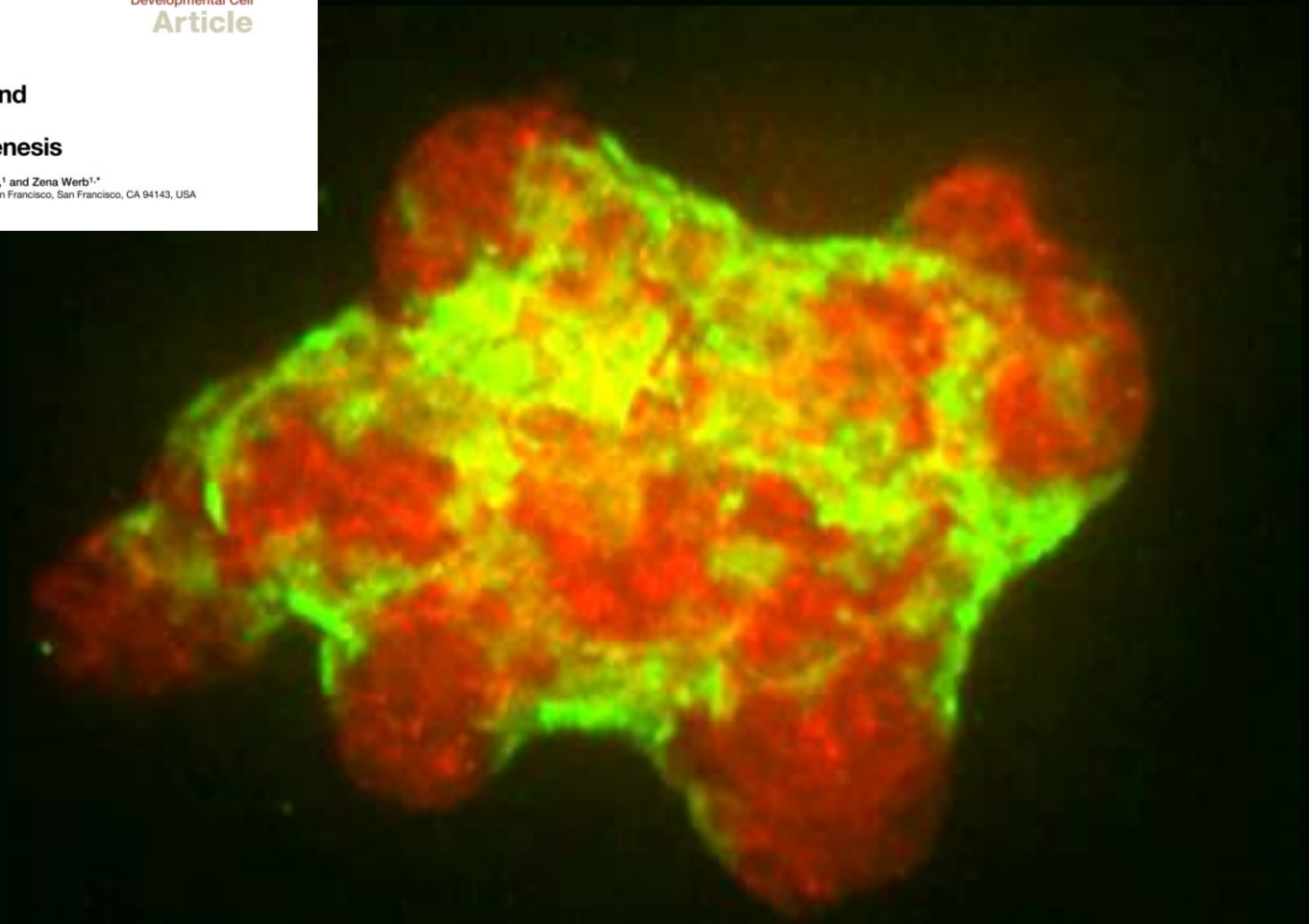

Matrigel
Collagen I
Branching morphogenesis
Transient protrusive migration and dissemination

Cell PRESS

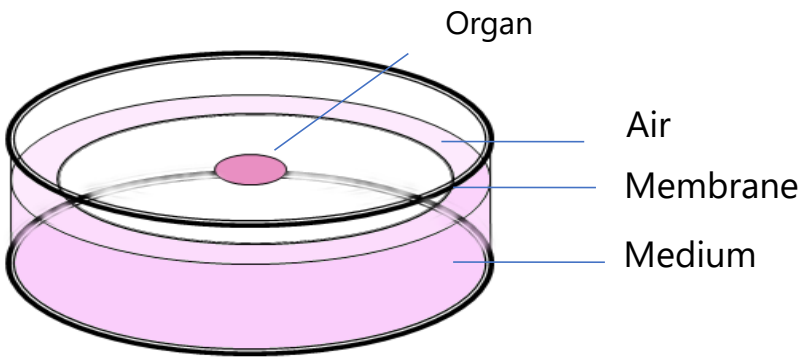
Developmental Cell
Article

Collective Epithelial Migration and Cell Rearrangements Drive Mammary Branching Morphogenesis

Andrew J. Ewald,[1,*] Audrey Brenot,[1] Myhanh Duong,[1] Bianca S. Chan,[1] and Zena Werb[1,*]
[1]Department of Anatomy and Program in Cell Biology, University of California, San Francisco, San Francisco, CA 94143, USA
*Correspondence: andrew.ewald@ucsf.edu (A.J.E.), zena.werb@ucsf.edu (Z.W.)
DOI 10.1016/j.devcel.2008.03.003

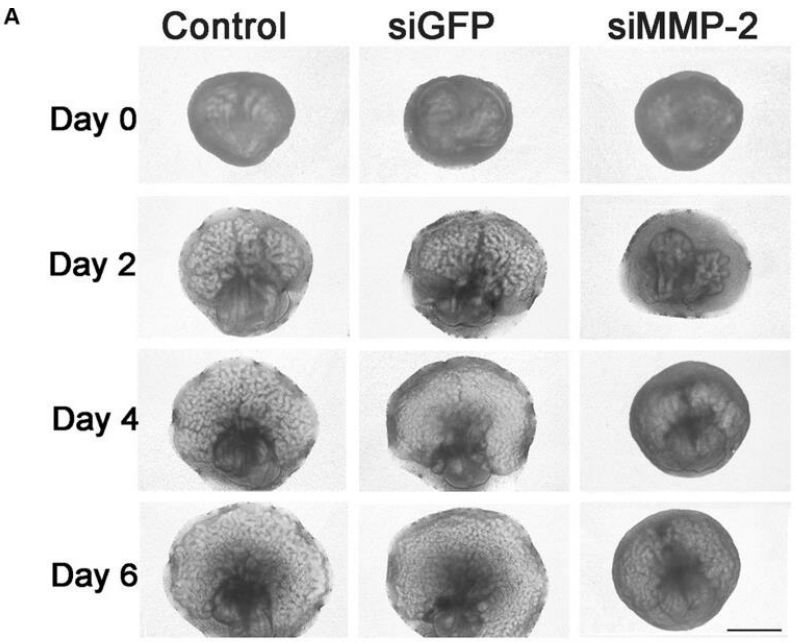


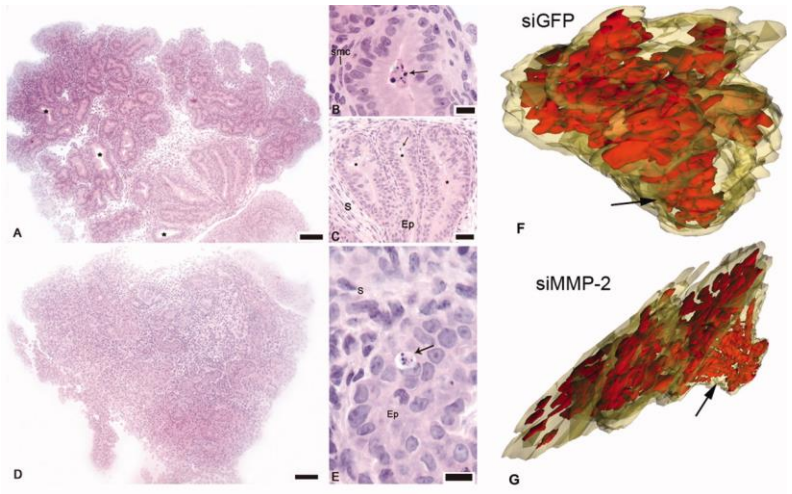
Whole organ (or organ slice) culture



MMP-2 Regulates Rat Ventral Prostate Development In Vitro

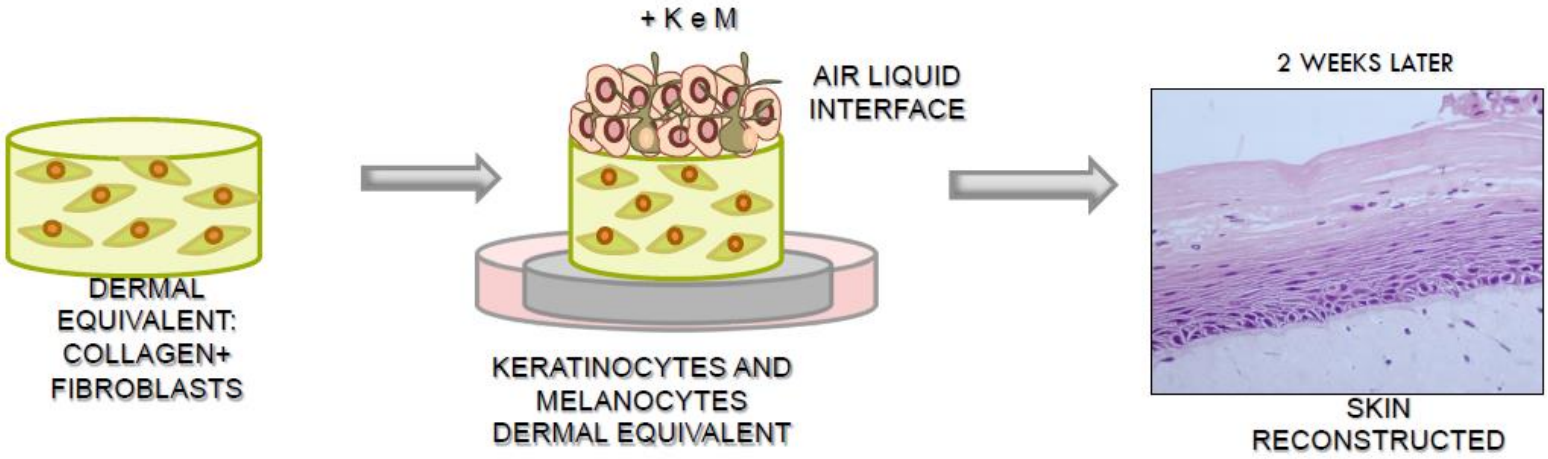
Alexandre Bruni-Cardoso,[1] Rafaela Rosa-Ribeiro,[1] Vinicius D. B. Pascoal,[2] Andre A. De Thomaz,[3] Carlos L. Cesar,[3,4] and Hernandes F. Carvalho[1,4]*

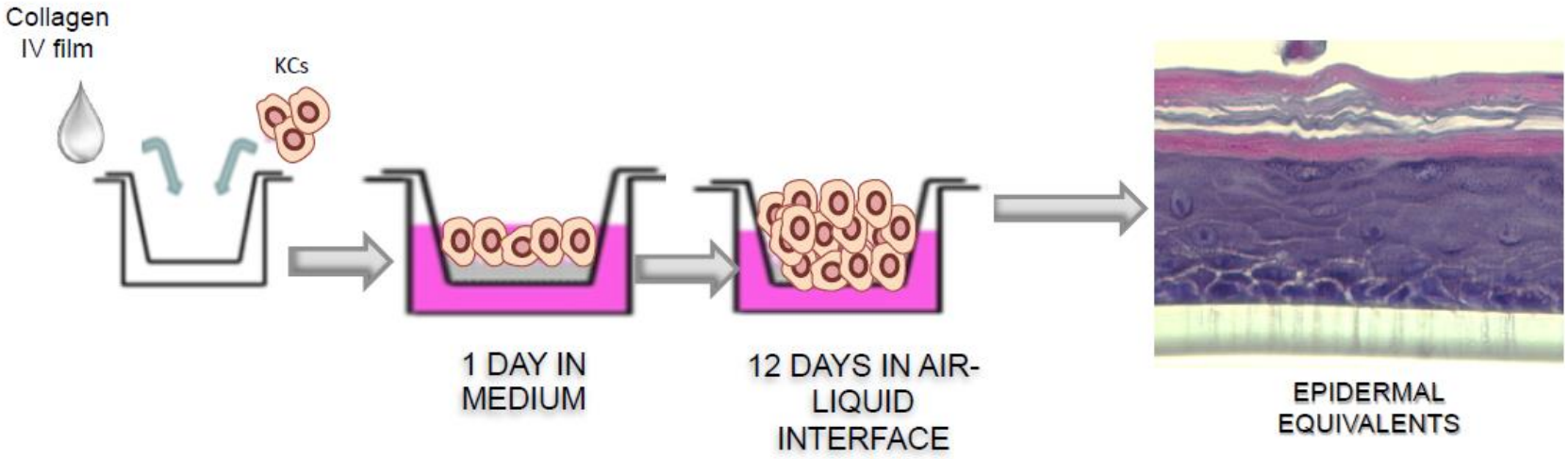




Bruni-Cardoso et al., 2010, Dev Dyn

3D models for skin

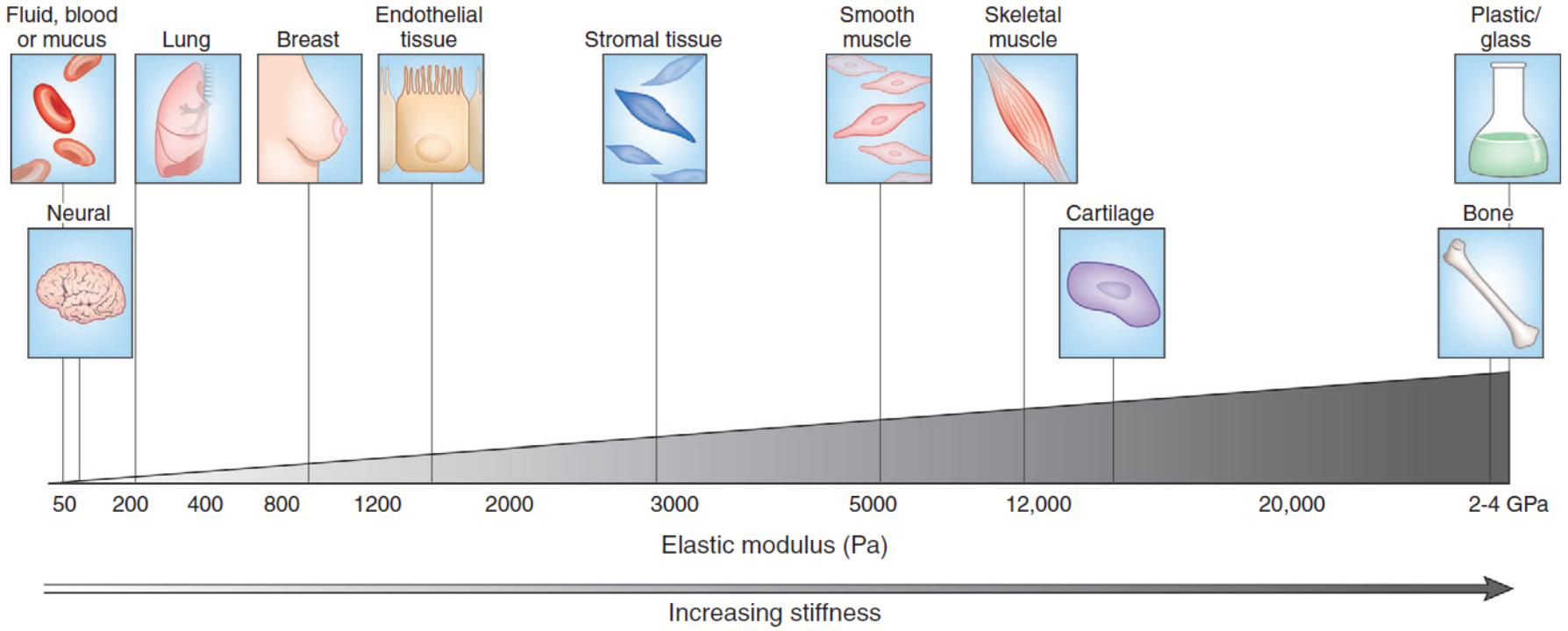




Used for testing cosmetics
Melanoma model

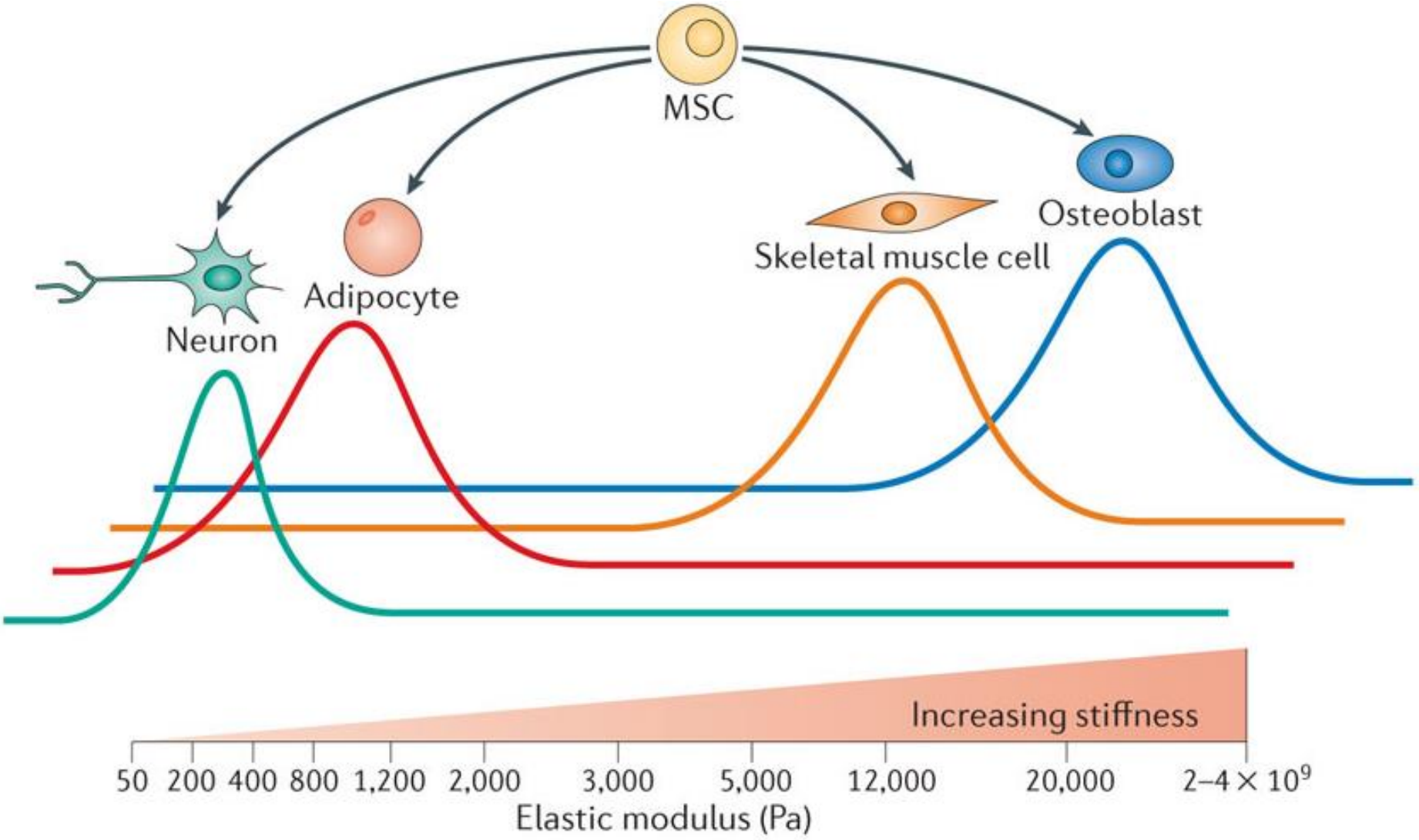
Silvya Stuchi Maria-Engler (FCF-USP)

Stiffeness matters!



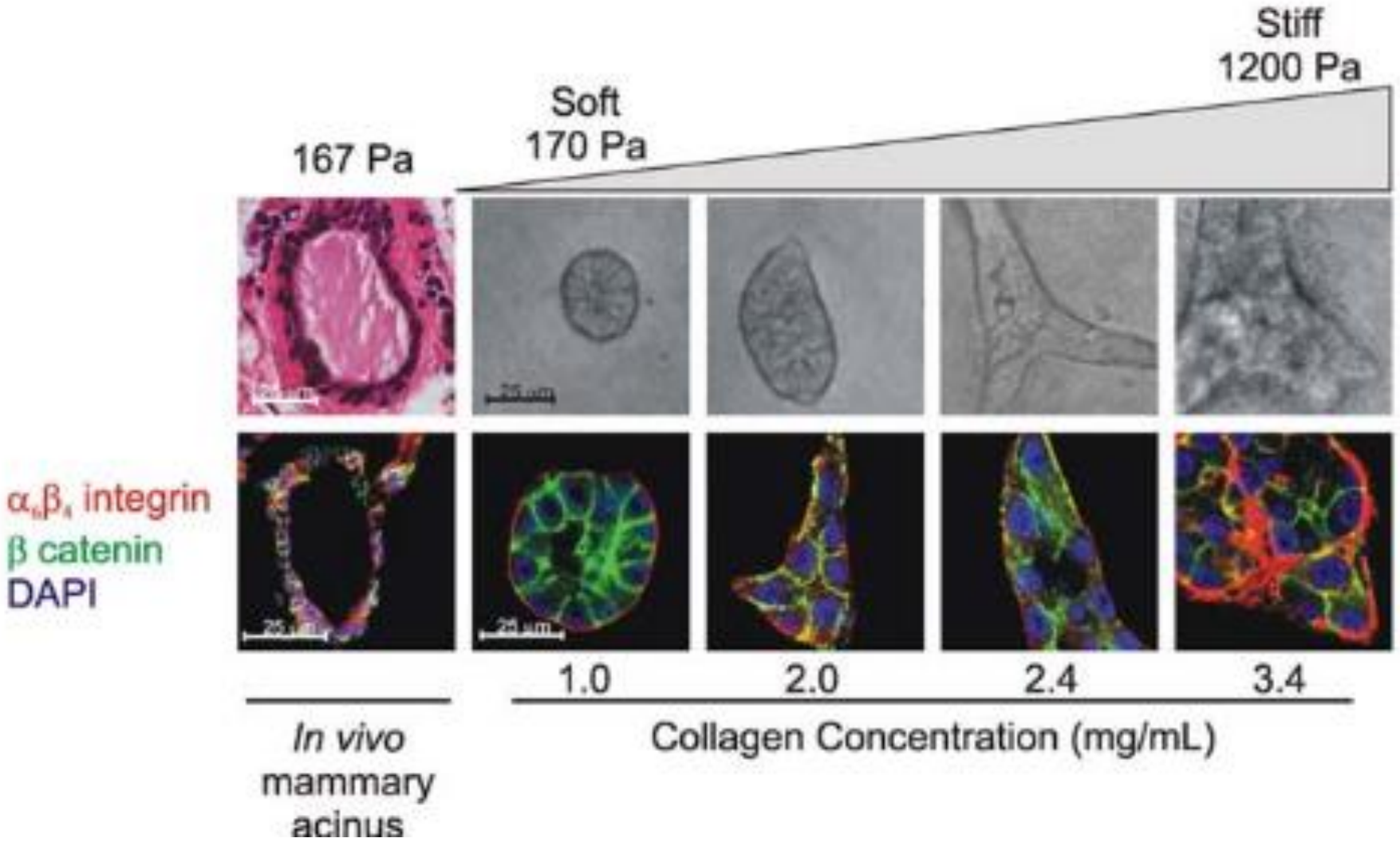
Cox and Erler (2011), Disease Models & Mechanisms

The ECM stiffness influences cell fate



Schematic from Halder et a., (2012 Nat. Rev. Mol. Cell Biol.). Based on Watt et al. PNAS (1988), McBeath et al. Dev. Cell (2004), Engler, et al, Cell (2006)

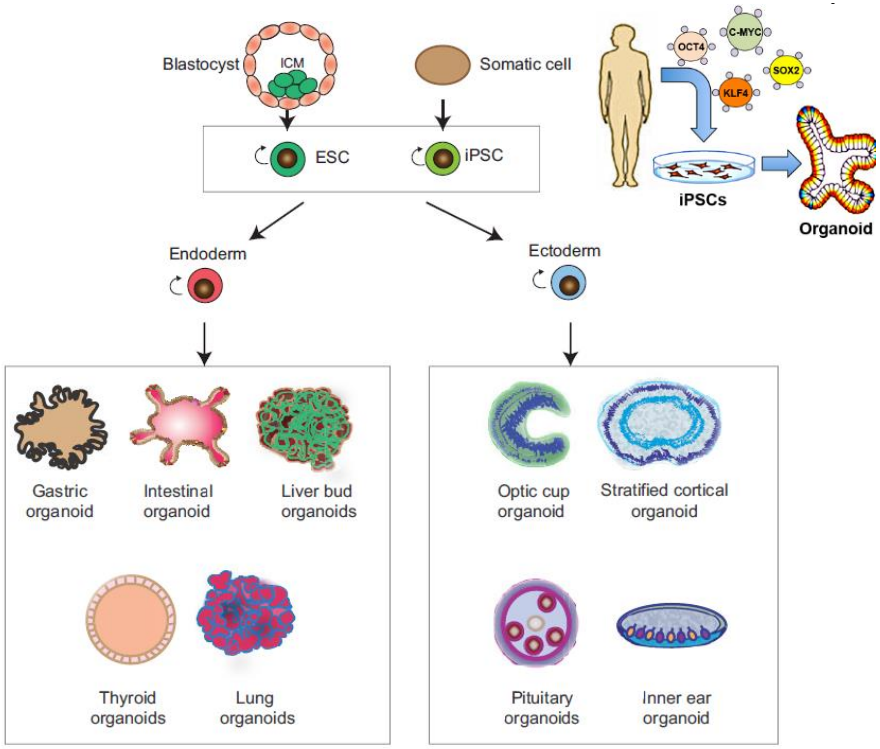
Physical properties of the ECM modulates malignant transformation in 3D cultures



Pazsek et al., Cancer Cell (2005)

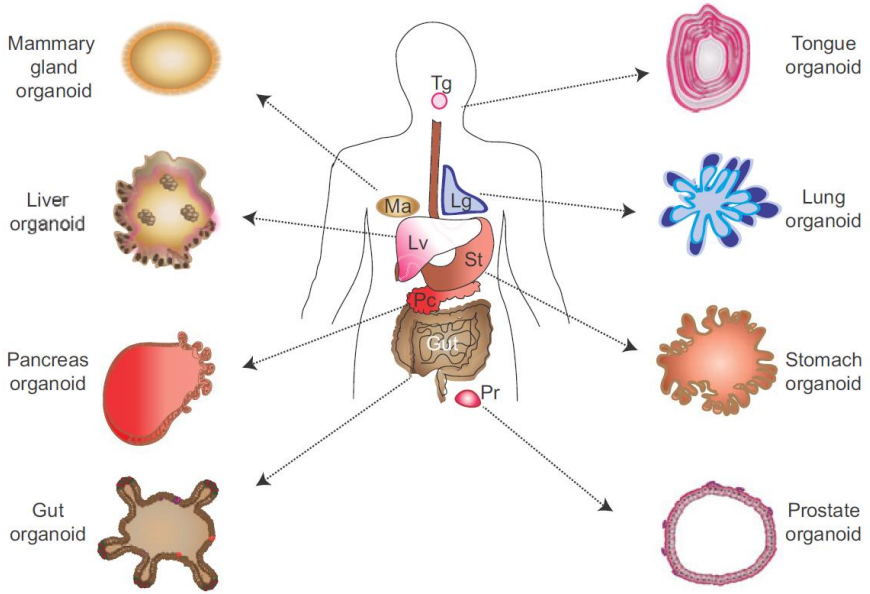
Stem cell-derived organoids

Pluripotent stem cell (PSC)-derived organoids.
PSCs [embryonic stem cells (ESCs) or induced PSCs (iPSCs)]

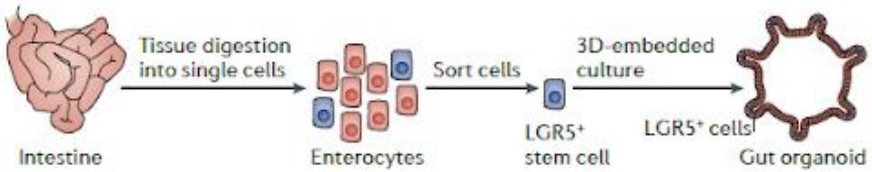


Huch and Koo, 2015, Development

Adult stem cell (AdSC)-derived organoids



Huch and Koo, 2015, Development



Shamir and Ewald, 2014, Nature Reviews – Molecular Cell Biology

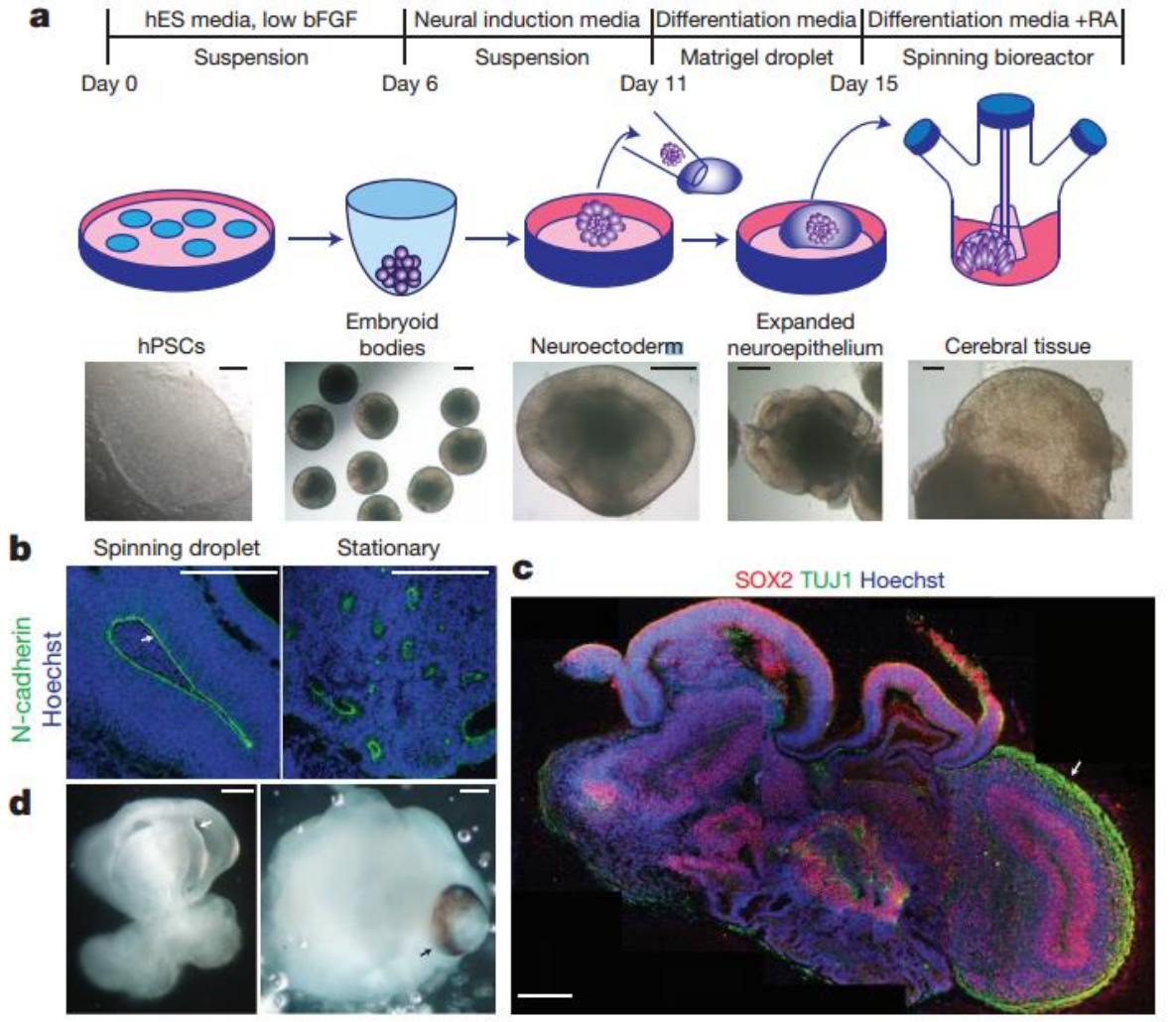
ARTICLE

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

Madeline Lancaster

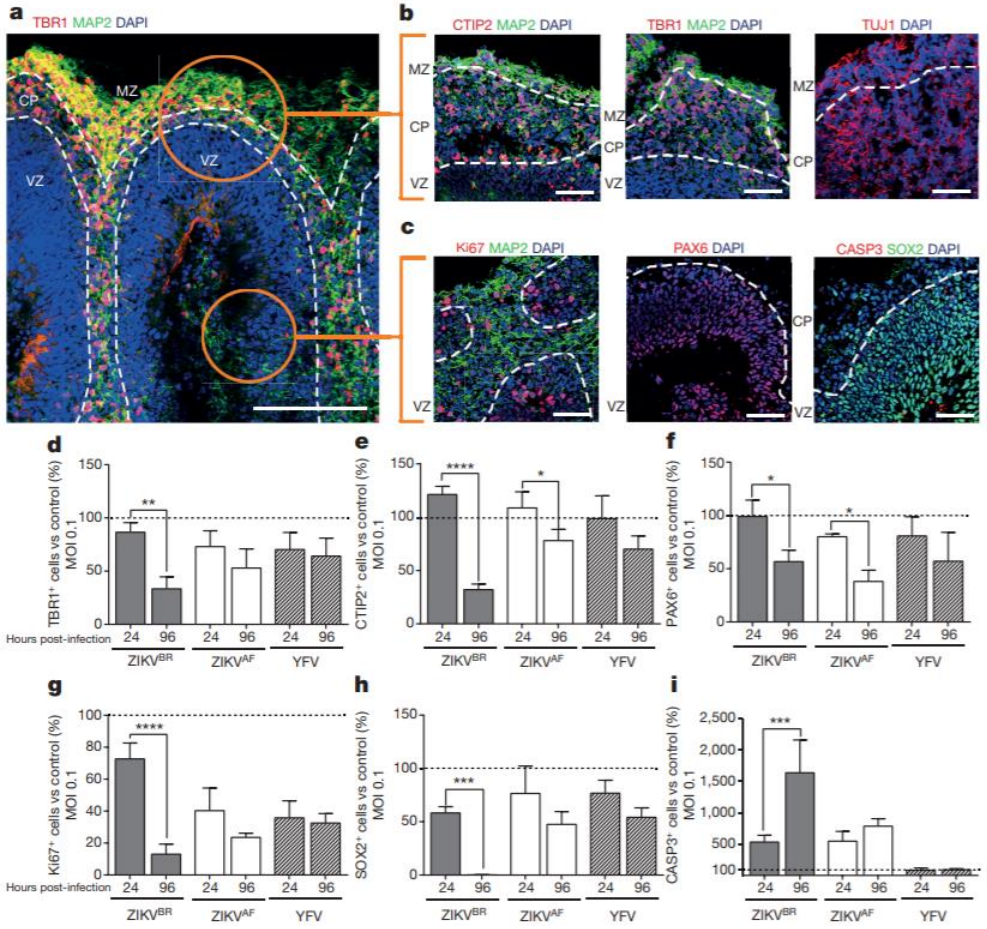


LETTER

doi:10.1038/nature18296

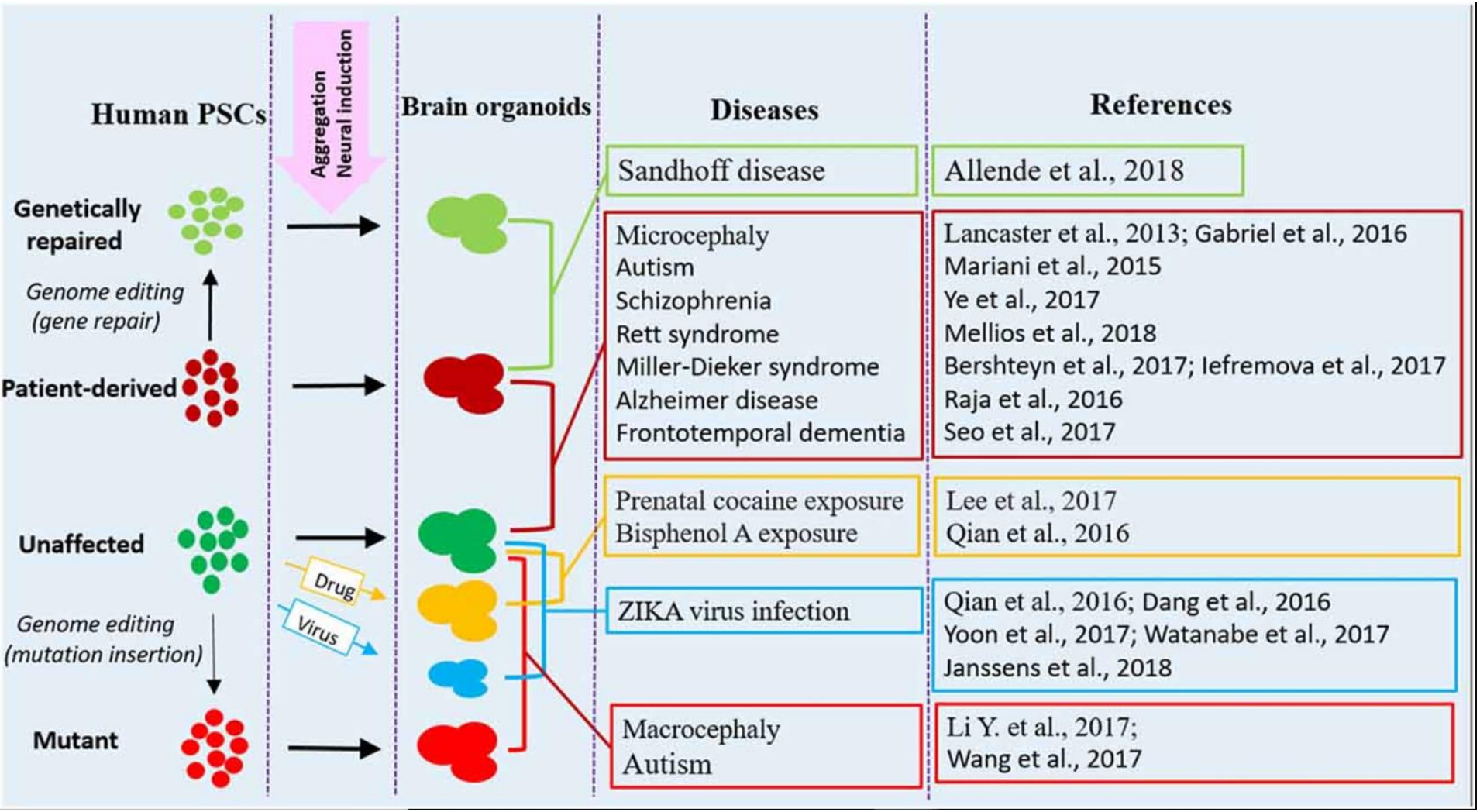
The Brazilian Zika virus strain causes birth defects in experimental models

Fernanda R. Cugola[1]*, Isabella R. Fernandes[1,2]*, Fabiele B. Russo[1,3]*, Beatriz C. Freitas[2], João L. M. Dias[1], Katia P. Guimarães[1], Cecília Benazzato[1], Nathalia Almeida[1], Graciela C. Pignatari[1,3], Sarah Romero[2], Carolina M. Polonio[4], Isabela Cunha[4], Carla L. Freitas[4], Wesley N. Brandão[4], Cristiano Rossato[4], David G. Andrade[4], Daniele de P. Faria[5], Alexandre T. Garcez[5], Carlos A. Buchpigel[5], Carla T. Braconi[6], Erica Mendes[6], Amadou A. Sall[7], Paolo M. de A. Zanotto[6], Jean Pierre S. Peron[4], Alysson R. Muotri[2] & Patricia C. B. Beltrão-Braga[1,8]



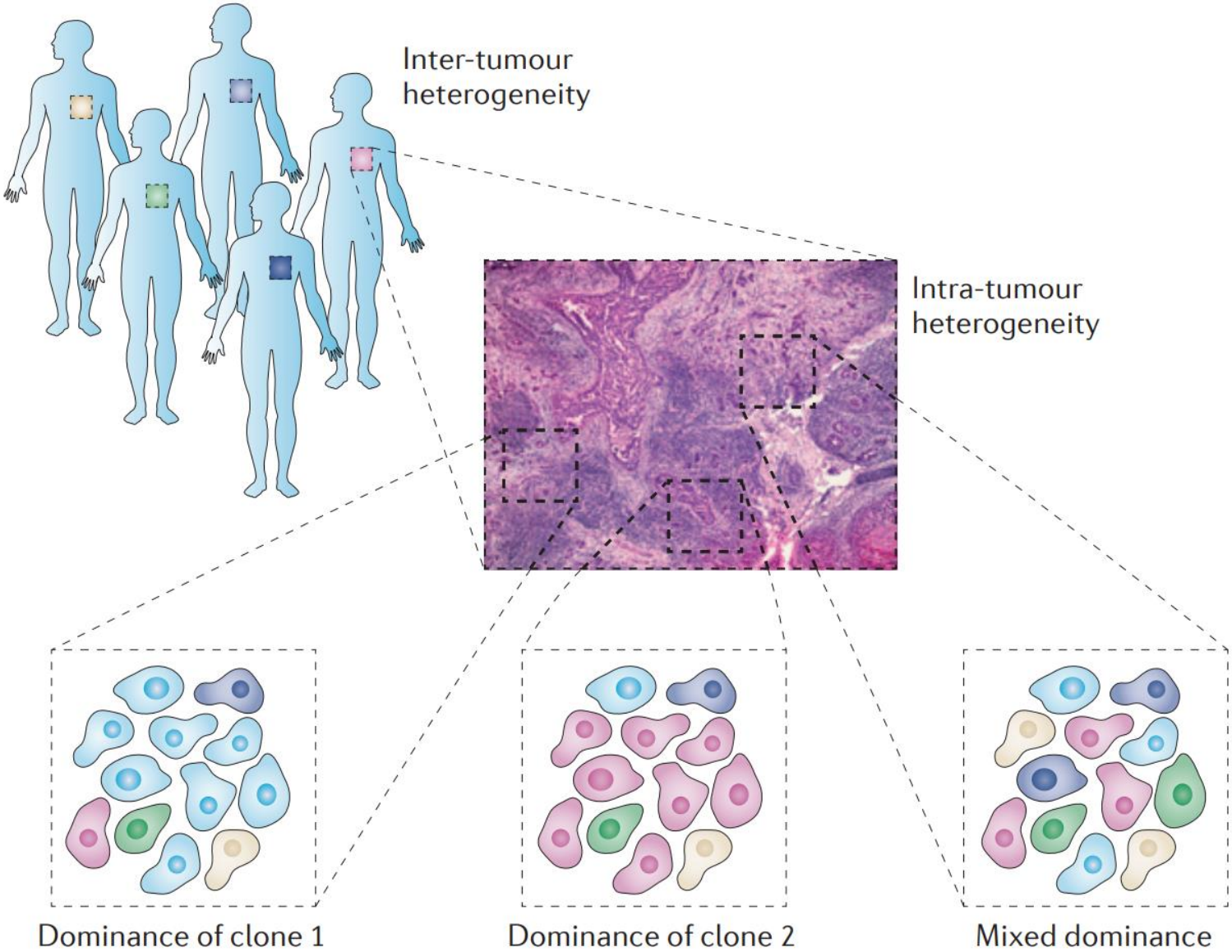
Cugola et al., 2016

Modeling Neurological Diseases With Human Brain Organoids



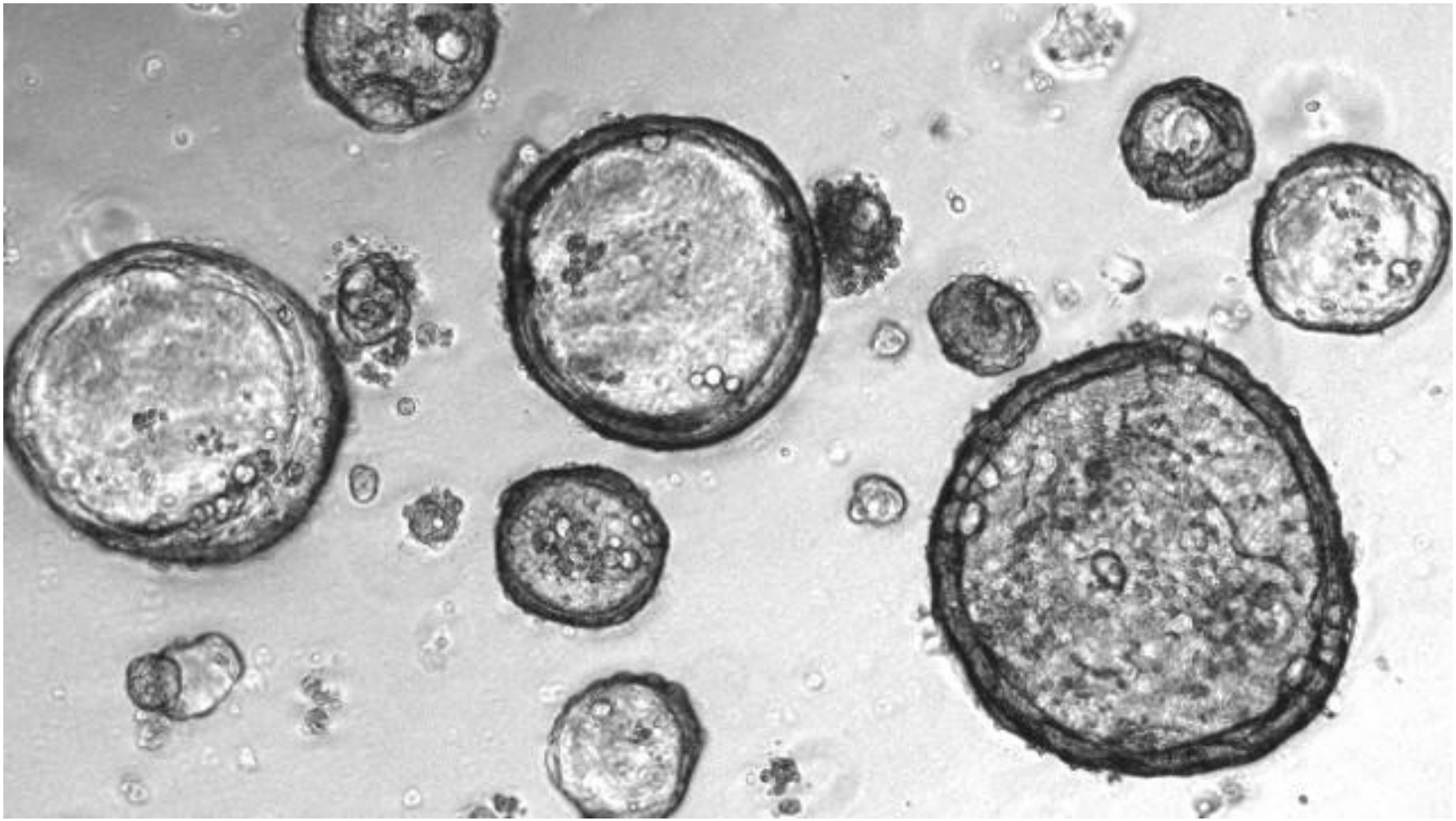
Wang, 2018, Front Synaptic Neurosci.

Cancer Heterogeneity



Marusyk et al., 2012

Patient-Derived Organoids (PDO)



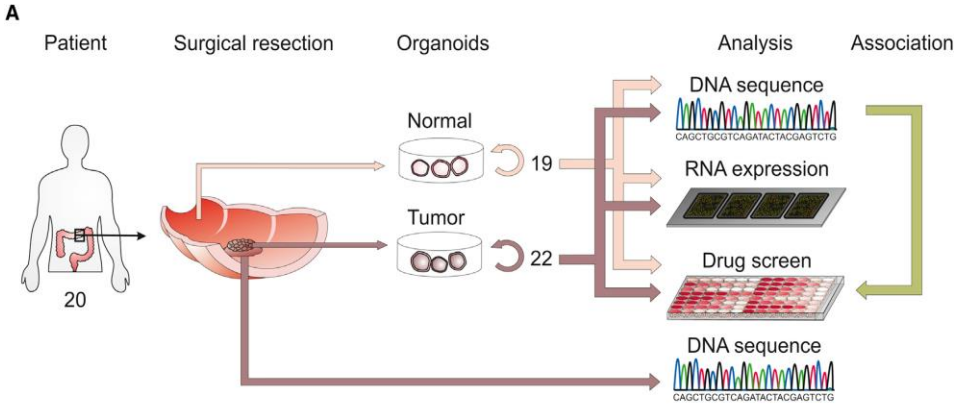
Gastroesophageal cancer organoids
GEORGE VLACHOGIANNIS

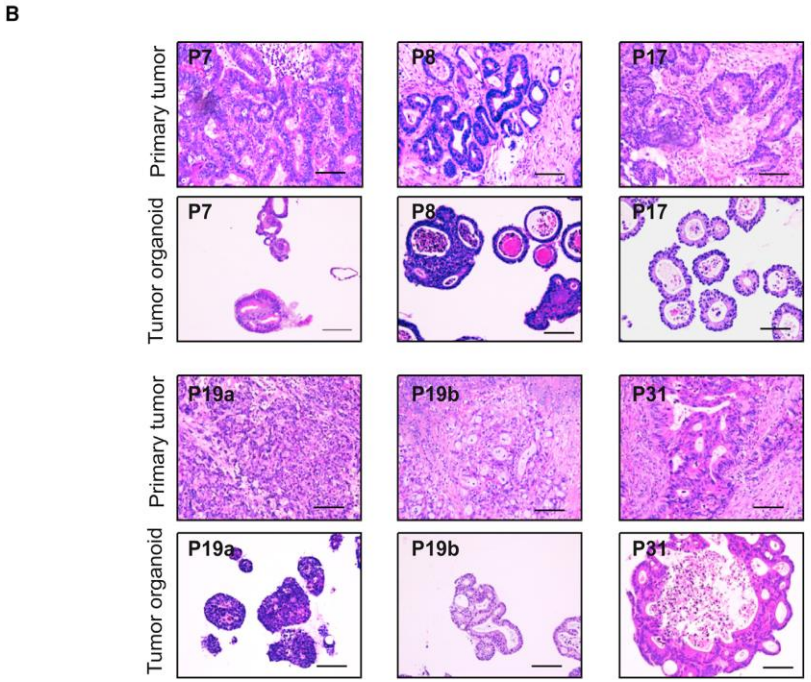
https://www.the-scientist.com/news-opinion/tumor-organoids-predict-how-well-patients-respond-to-cancer-drugs-30086

Resource

Cell

Prospective Derivation of a Living Organoid Biobank of Colorectal Cancer Patients





Wetering et al, 2015, Cell

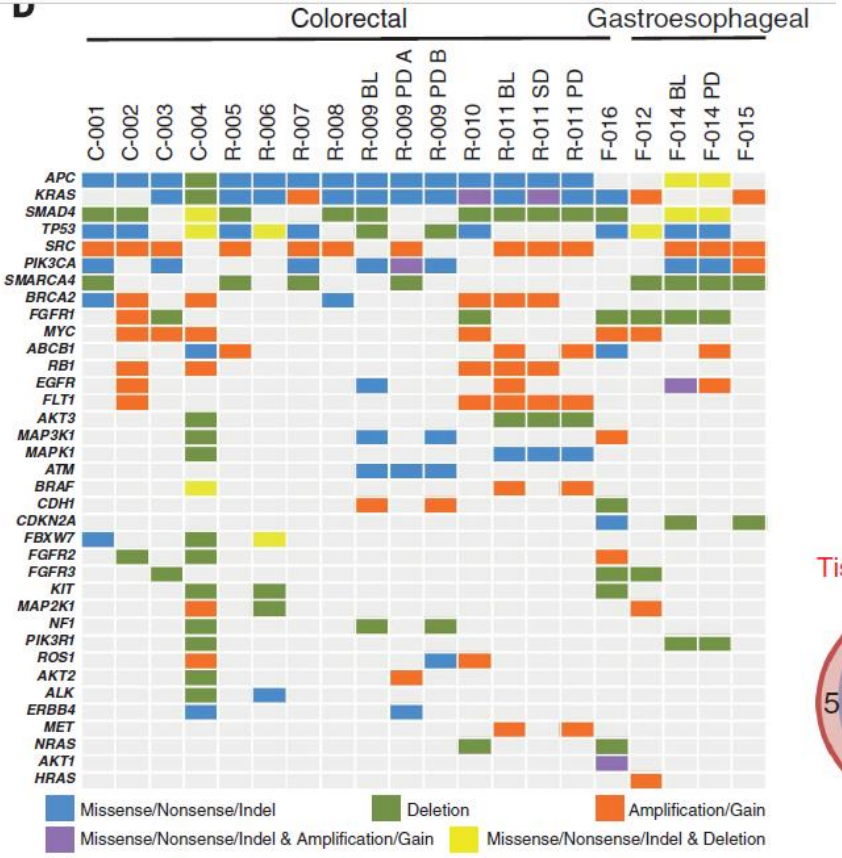
Corrected 20 February 2018. See full text.

RESEARCH

ORGANOIDS

Patient-derived organoids model treatment response of metastatic gastrointestinal cancers

Georgios Vlachogiannis,[1] Somaieh Hedayat,[1] Alexandra Vatsiou,[2] Yann Jamin,[3] Javier Fernández-Mateos,[1,2] Khurum Khan,[1,4] Andrea Lampis,[1] Katherine Eason,[1] Ian Huntingford,[1] Rosemary Burke,[5] Mihaela Rata,[3] Dow-Mu Koh,[3,6] Nina Tunariu,[3,6] David Collins,[3] Sanna Hulkki-Wilson,[1] Chanthirika Ragulan,[1] Inmaculada Spiteri,[2] Sing Yu Moorcraft,[4] Ian Chau,[4] Sheela Rao,[4] David Watkins,[4] Nicos Fotiadis,[6] Maria Bali,[3,6] Mahnaz Darvish-Damavandi,[1] Hazel Lote,[1,4] Zakaria Eltahir,[1] Elizabeth C. Smyth,[4] Ruwaida Begum,[4] Paul A. Clarke,[5] Jens C. Hahne,[1] Mitchell Dowsett,[7] Johann de Bono,[8] Paul Workman,[5] Anguraj Sadanandam,[1] Matteo Fassan,[9] Owen J. Sansom,[10] Suzanne Eccles,[5] Naureen Starling,[4] Chiara Braconi,[4,5] Andrea Sottoriva,[2] Simon P. Robinson,[3] David Cunningham,[4] Nicola Valeri[1,4]*



G

		Viability in response to palbociclib		
		<60%	≥60%	Total
RB1 Log2R	>0.6	4	0	4
	≤0.6	2	12	14
	Total	6	12	18

P<0.005

Vlachogiannis, et al 2018. Science

Table I. Characterization of Different Cancer Models[a]

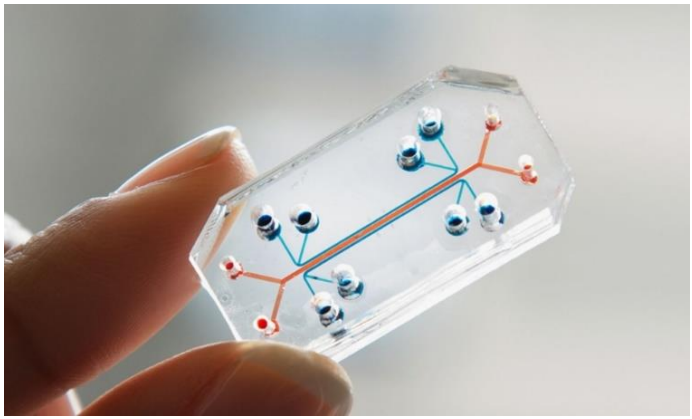
Features	Cell lines	PDXs	Organoids
Success rate of initiation	•	••	•••
Expansion	•••	•	••
Cancer subtype modeling	•	–	•••
Biological stability	•	••	••
Genetic manipulation	•••	–	•••
High-throughput drug screening	•••	–	••
Low-throughput drug screening	•••	•	•••
Ease of downstream assays	•••	•	•••
Cost benefits	•••	–	••
Time consumption for modeling	•	•••	•
Ease of maintenance	•••	–	••

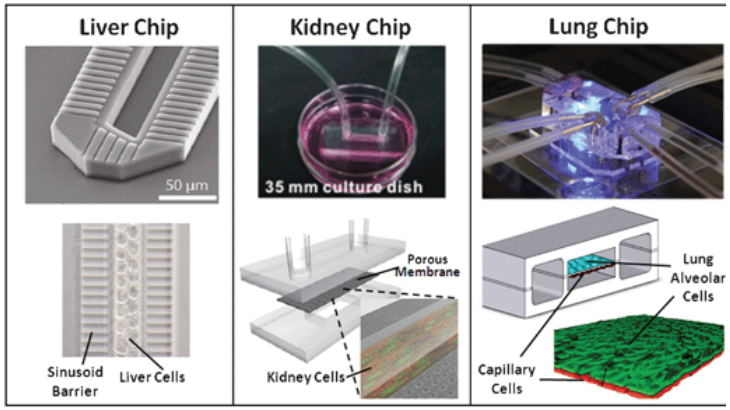
Adapted, with permission, from [29].
[a]•••, Best; ••, suitable; •, possible; and –, unsuitable.

Aboulkheyr et al 2018, Trends in Biotechnology

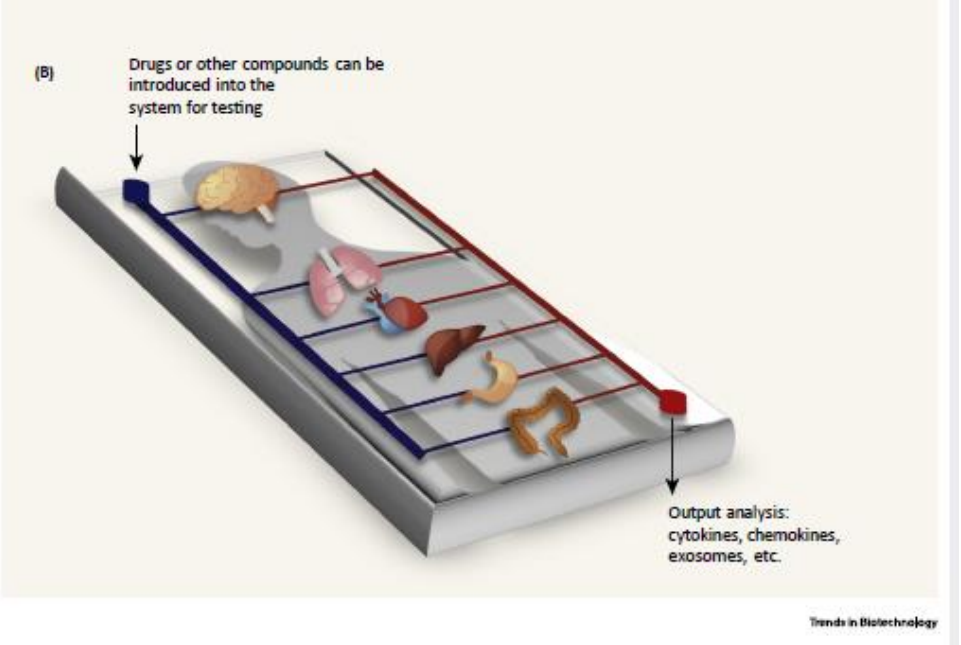
What´s next?

Organs on a chip





Humans on a chip



Aboulkheyr et al 2018, Trends in Biotechnology

“You are as good as your model”

Mina J. Bissell

“There is no perfect model”

Arthur Lander (UC Irvine)

3D cell culture does not require fancy equipment

- Cell culture hood (i.e., laminar-flow hood or biosafety cabinet)
- Incubator (humid CO2 incubator recommended)
- Water bath
- Centrifuge
- Refrigerator and freezer (–20°C)
- Cell counter (e.g., hemacytometer)
- Inverted microscope
- Liquid nitrogen (N2) freezer or cryostorage container
- Sterilizer (i.e., autoclave)
- Cell culture vessels (e.g., flasks, Petri dishes, roller bottles, multi-well plates)
- Pipettes and pipettors
- Syringes and needles
- Waste containers
- Media, sera, and reagents

Literature and resources

http://jcs.biologists.org/content/130/1

http://dev.biologists.org/content/144/6

Aboulkheyr et al., Trends in Biotechnology, 2018 doi:https://doi.org/10.1016/j.tibtech.2017.12.005

Kevala and Lancaster, Dev Biol, 2016, https://doi.org/10.1016/j.ydbio.2016.06.037

Kelava and Lancaster, Cell Stem Cell. 2016 VOLUME 18, ISSUE 6, P736-748 doi :https://doi.org/10.1016/j.stem.2016.05.022

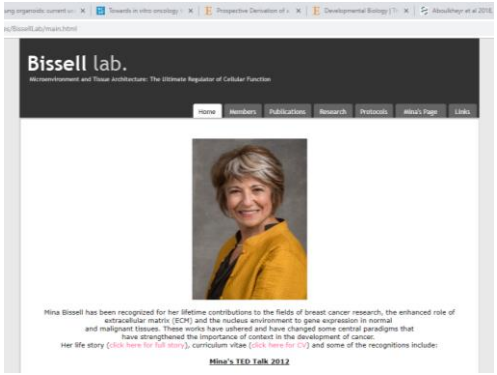
Shamir and Ewald, Nat Rev Mol Cell Biol. 2014 Oct;15(10):647-64. doi: 10.1038/nrm3873

Simian and Bissell, J Cell Biol. 2017 Jan 2;216(1):31-40. doi: 10.1083/jcb.201610056

Wetering et al., Cell. 2015 May 7;161(4):933-45. doi: 10.1016/j.cell.2015.03.053.

Protocols

Bissell lab http://www2.lbl.gov/LBL-Programs/lifesciences/BissellLab/main.html



Joan Brugge Lab https://brugge.med.harvard.edu/

Acknowledgments

Instituto de Química

e-signal lab

Lab members

Post-doc

Ana Zen Fiore

Rebeka Tomasin

Grad Students

Ana Maria

Antonio Manucci

Pedro Ribeiro

Mayara Botelho

Giovani Genesi

The e-signal lab is currently funded by:

FAPESP

CNPq

Conselho Nacional de Desenvolvimento Científico e Tecnológico

serrapilheira

email: brunicar@iq.usp.br

homepage: www.ecm-signaling.com