Organoids: A historical perspective of thinking in three dimensions

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Instituto de Nanosistemas
Universidad Nacional de San Martín
Buenos Aires-Argentina

Organs-on-a-chip…the new revolution in preclinical studies

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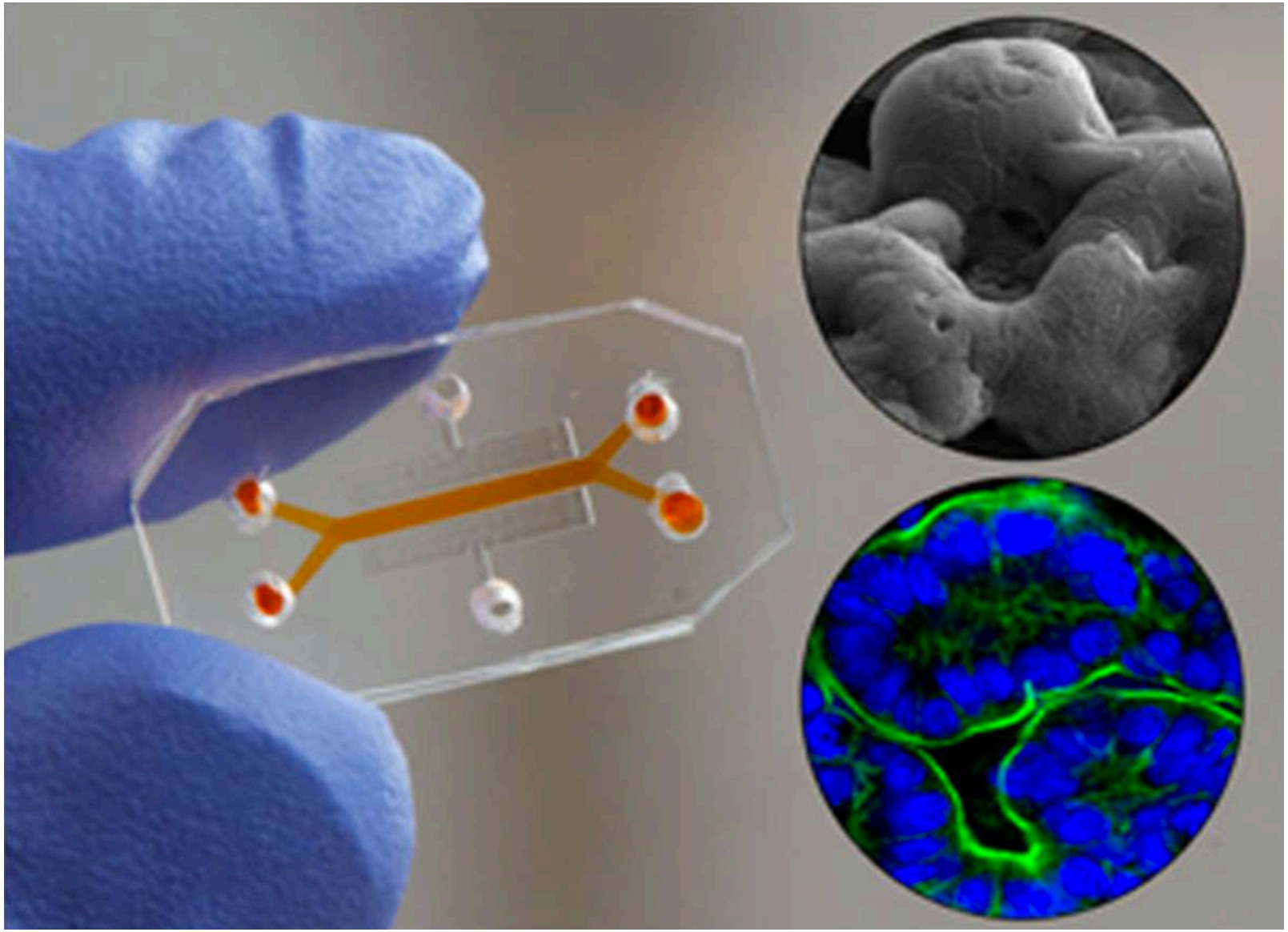
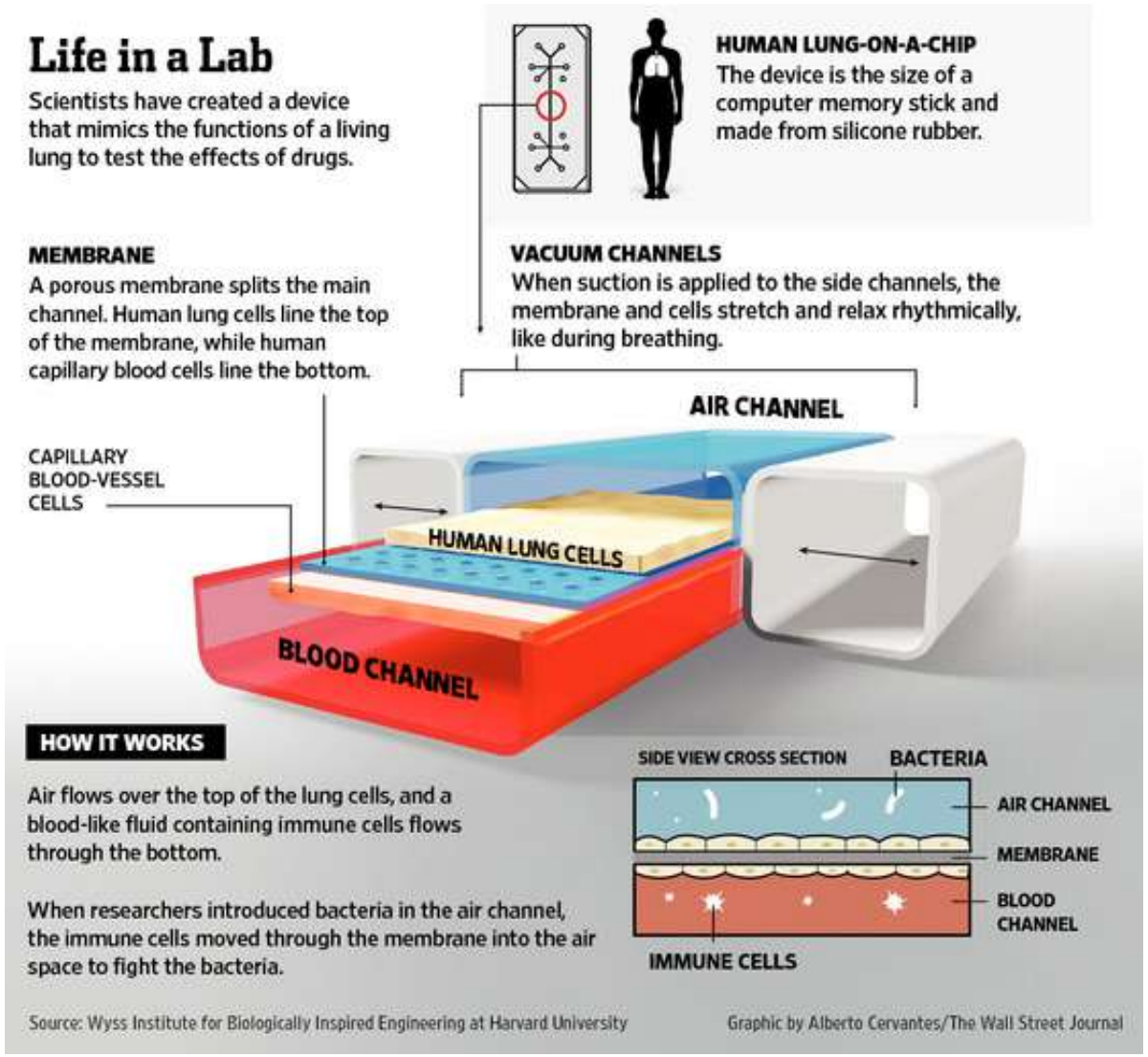
FDA Collaborates with Emulate to Use Organs-on-Chips Technology as a Toxicology Testing Platform

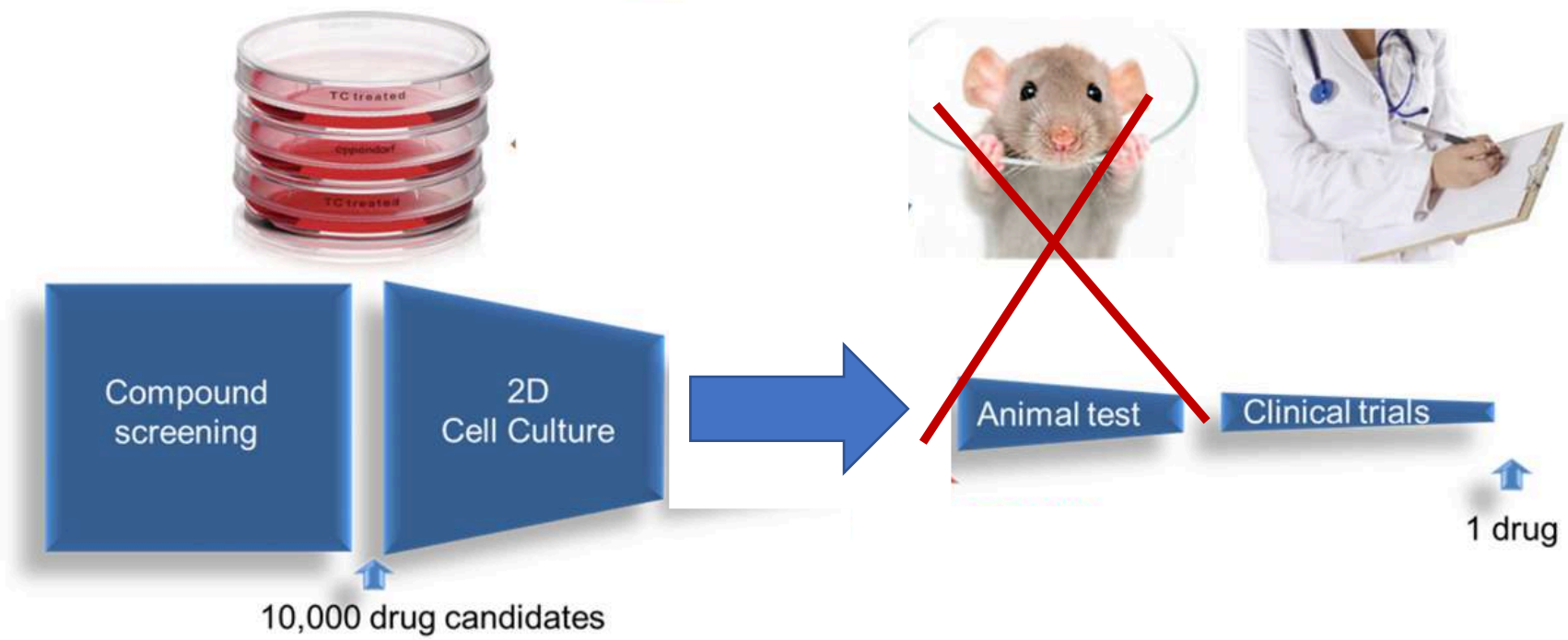
April 12, 2017





Source: Wyss Institute for Biologically inspired Engineering at Harvard University

Current drug development pathway



How did we get here?

JCB: Perspective

Organoids: A historical perspective of thinking in three dimensions

Marina Simian[1] and Mina J. Bissell[2]

[1]Instituto de Nanosistemas, Universidad Nacional de San Martín, San Martín 1650, Provincia de Buenos Aires, Argentina
[2]Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

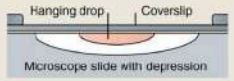
What is an organoid?

"…we define an organoid as a unit of function of a given organ that is able to reproduce, in culture, a biological structure similar in architecture and function to its counterpart in vivo. The origin of this unit is today multiple, as it can come from a fragment of tissue, a stem cell located in an adult organ, an embryonic stem cell, or an induced pluripotent stem cell. "

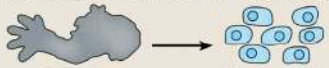
Simian & Bissell, 2017, JCB

Journey through the milestones of tissue culture that led to the development of organs on a chip…

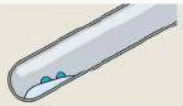
1906 Harrison
Hanging drop cell culture method



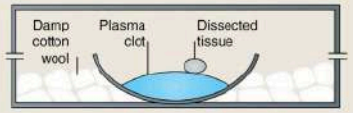
1914 Thomson
Definition of cultures as "controlled" vs "uncontrolled" when tissue organization and functionality are lost



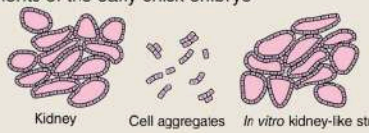
1926 Strangeways & Fell
Tube cultures



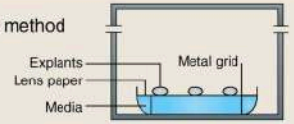
1929 Fell & Robison
Watch glass method



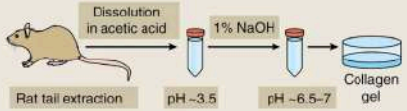
1952 Moscona & Moscona
Dissociation and aggregation of cells from organ rudiments of the early chick embryo



1954 Trowell
Grid cell culture method



1956 Ehrmann & Gey
Reconstituted collagen from rat tails



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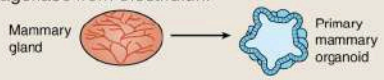
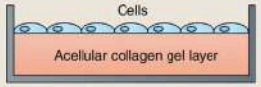
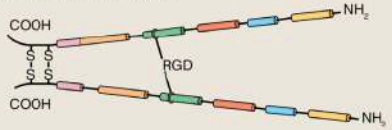
Dissociation of mammary glands into organoids using collagenase from *Clostridium*



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

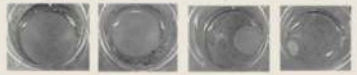


1973 Gahmberg & Hakomori; Ruoslahti et al.; Hynes
Discovery of Fibronectin



1975 Michalopoulos and Pitot
Differentiation of hepatocytes on floating collagen gels

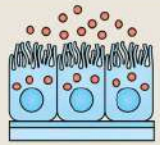
1977 Emerman and Pitelka
Culture of differentiated mammary epithelial cells on floating collagen gels



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

1979 Timpl et al.
Characterization of laminin

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.



1906
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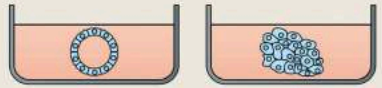
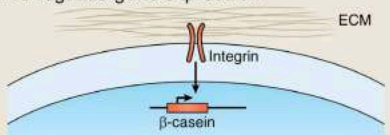
1984

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.

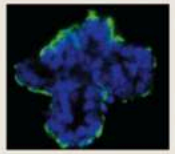
1989 Barcellos-Hoff et al. **1992** Petersen et al.
Use of a laminin-rich matrix to develop assays of mammary morphogenesis and to distinguish between healthy and malignant human epithelial cells.



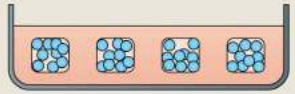
1991 Streuli et al.
Integrins regulate gene expression



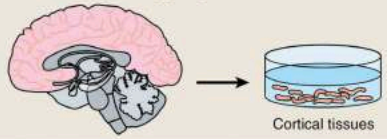
2001 Simian et al.
Use of 3D collagen cultures to study the mechanisms of mammary gland branching morphogenesis



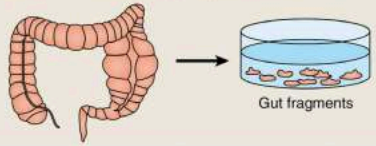
2006 Nelson et al.
Micropattern gels provide positional cues that establish the range of action of TGF-β in morphogenesis vs invasion



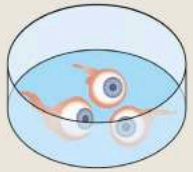
2008 Eiraku et al.
Self-organized formation of polarized cortical tissues from ESCs using 3D aggregation cultures



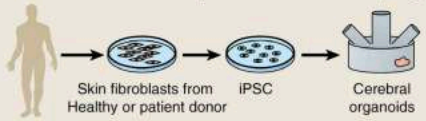
2009 Sato et al.
"Mini-guts": a culture system allows growth of epithelial organoids from a single Lgr5-positive stem cell



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
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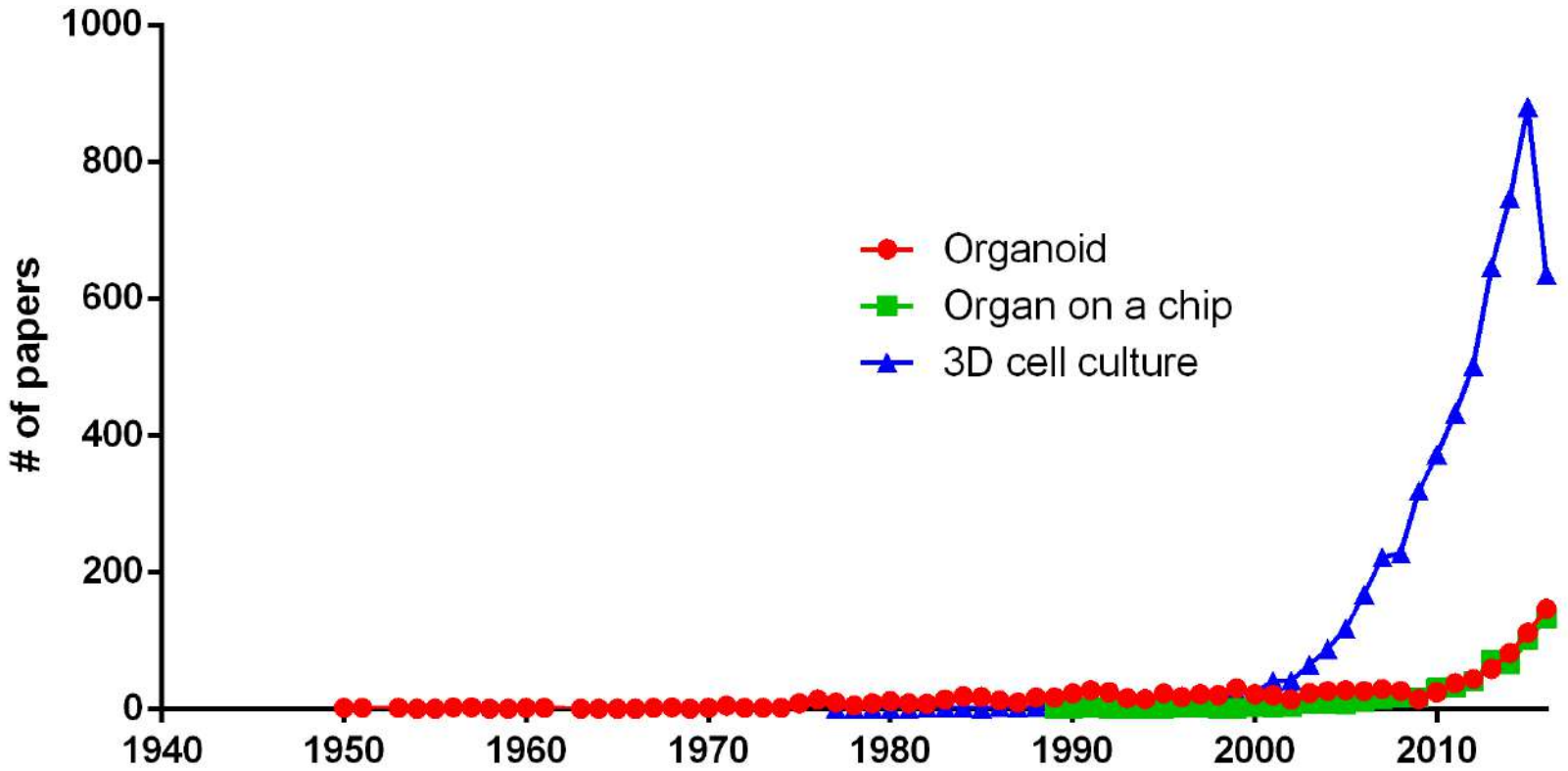
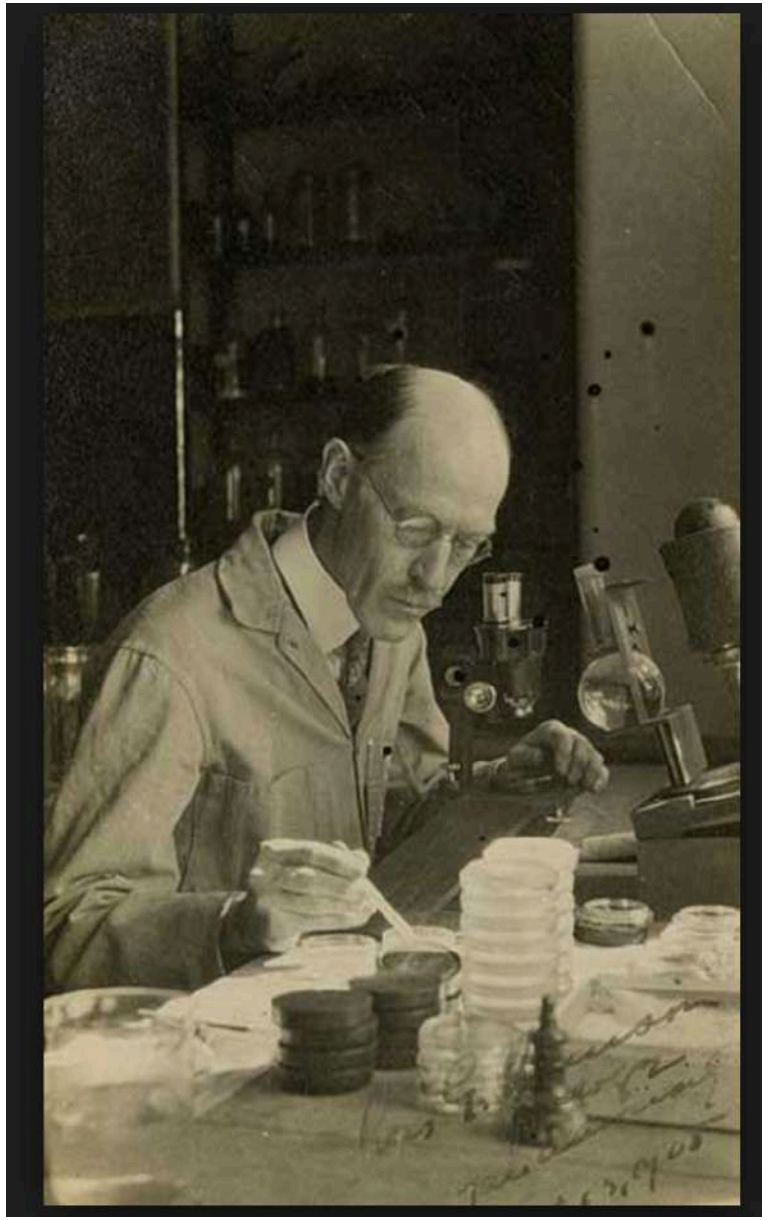
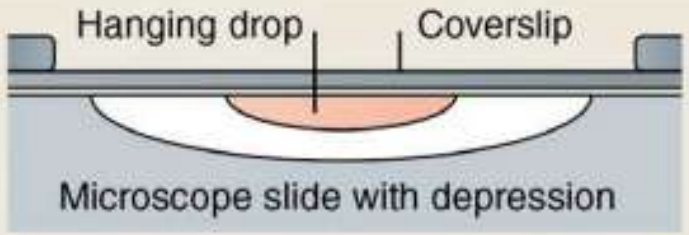
Pubmed citations per year

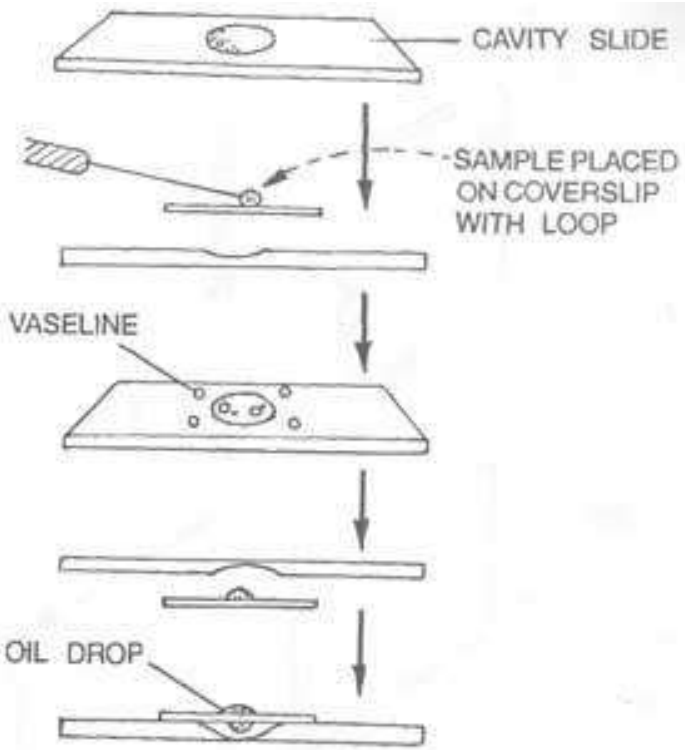


do we culture cells?



1906 Harrison
Hanging drop cell culture method





The Royal Society of Medicine.

Marcus Beck Laboratory Reports.—No. 2.

Some Further Researches on the Cultivation of Tissues *in vitro*.

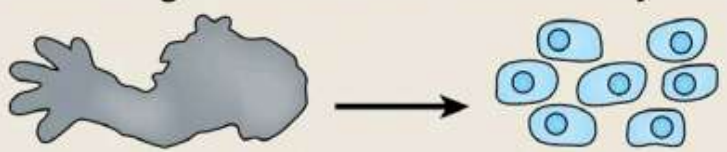
By David Thomson, M.B., Ch.B.Edin., D.P.H.Cantab.[1]

Read before a Meeting of the Society on May 11, 1914.

The President, Sir Francis H. Champneys, Bt., M.D., in the Chair.

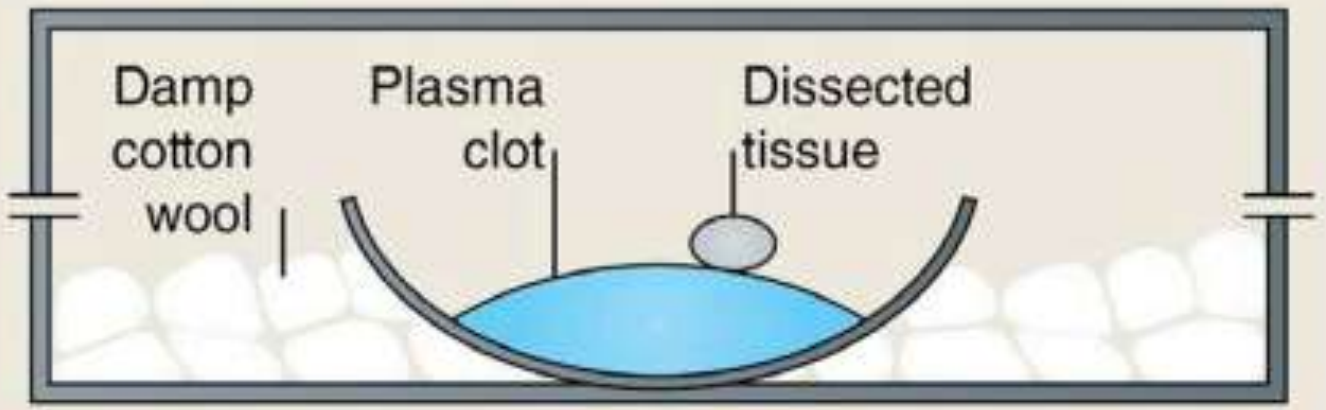
1914 Thomson

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



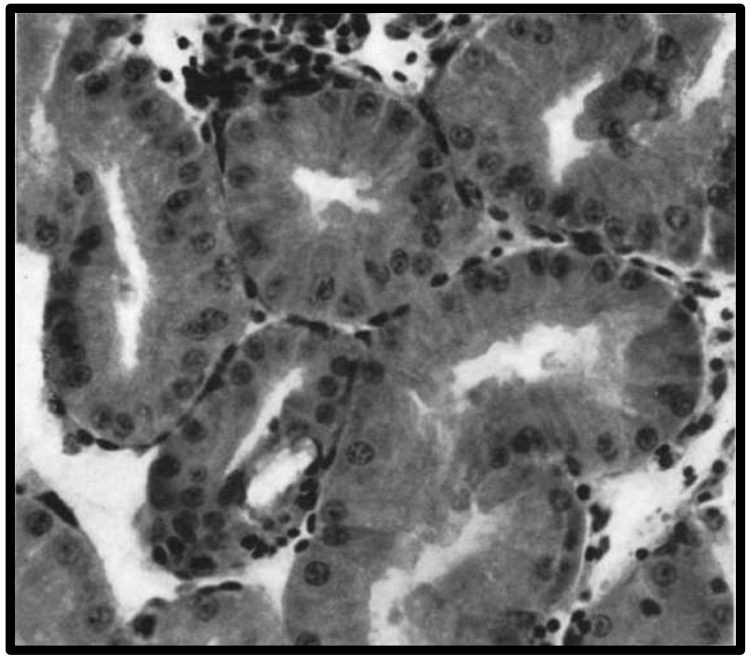
1929 Fell & Robison

Watch glass method

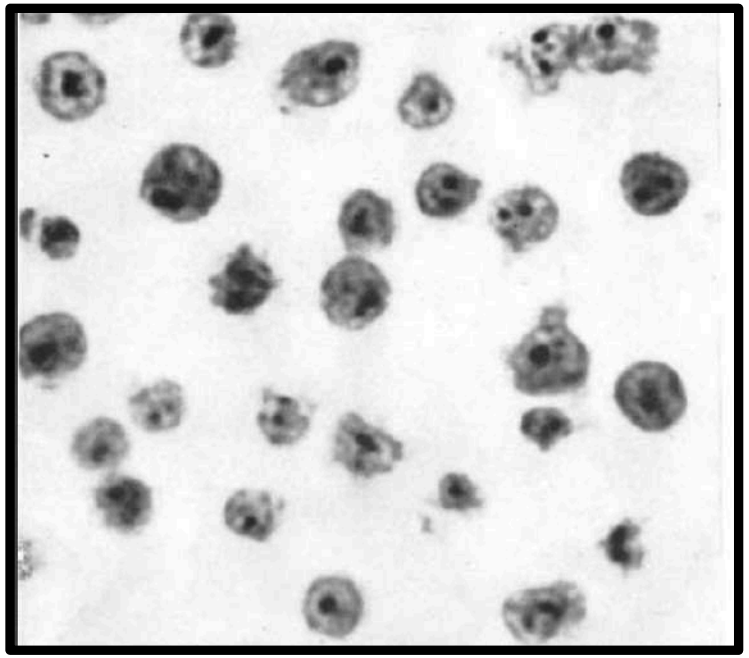


Moscona and Moscona, 1952

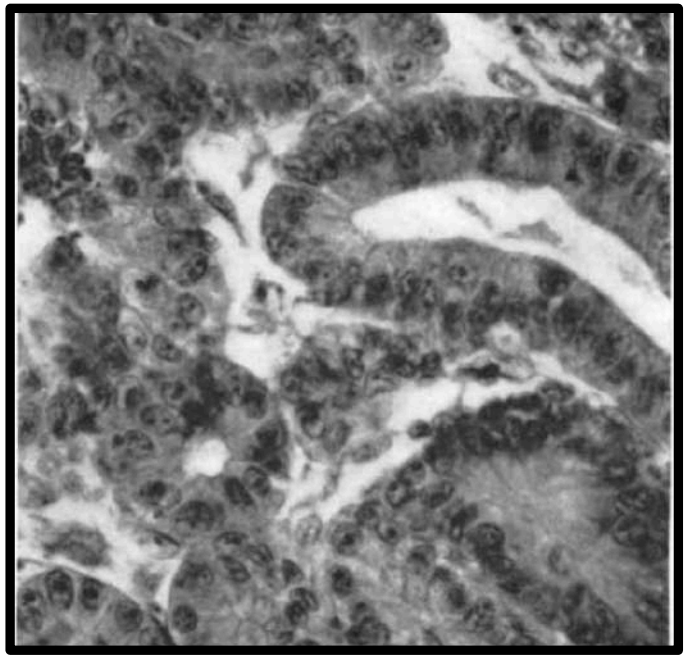
Dissociation and aggregation of cells from organ rudiments



Kidney



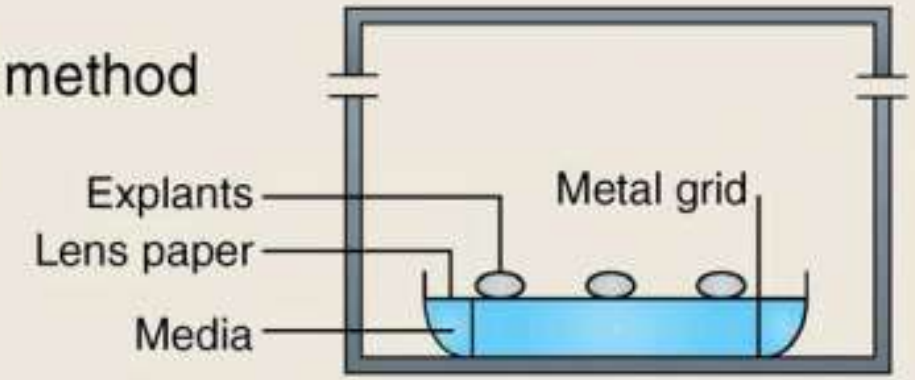
Cell aggregates

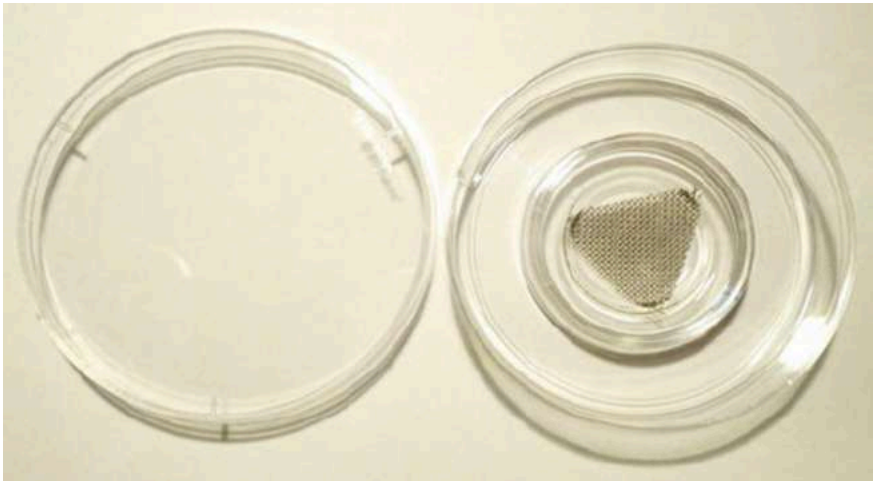


In vitro kidney-like structures

1954 Trowell

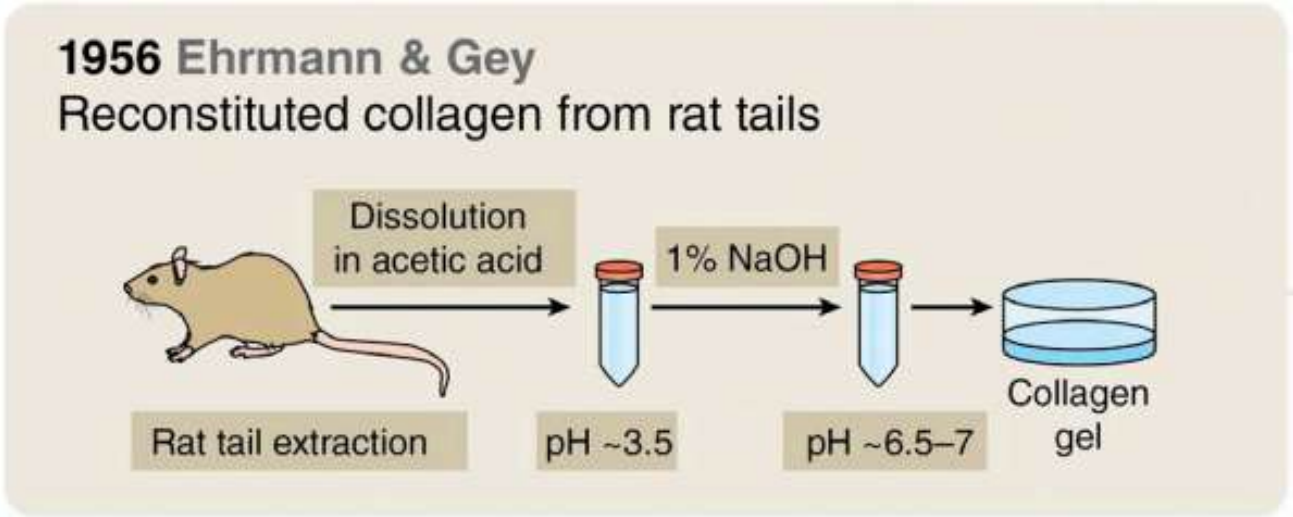
Grid cell culture method





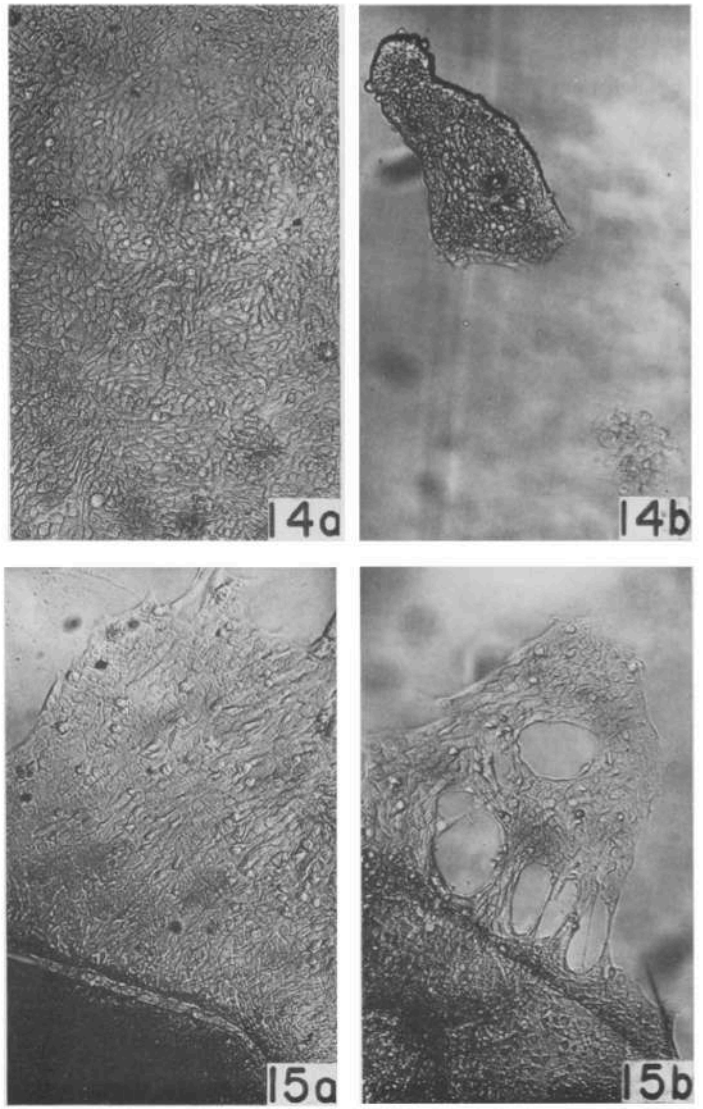
Use of collagen: 1956

- Huzella, T. 1932. Orientation de la croissance des cultures de tissus sur la trame fibrillaire artificielle coagulée de la solution de collagène. SAC r. Soc. Biol. Paris. 109:515.



The Growth of Cells on a Transparent Gel of Reconstituted Rat-Tail Collagen[1,2]

ROBERT L. EHRMANN[3] *and* GEORGE O. GEY,[4] *Division for Cellular Pathology, Department of Surgery, The Johns Hopkins Hospital and Medical School, Baltimore, Md.*



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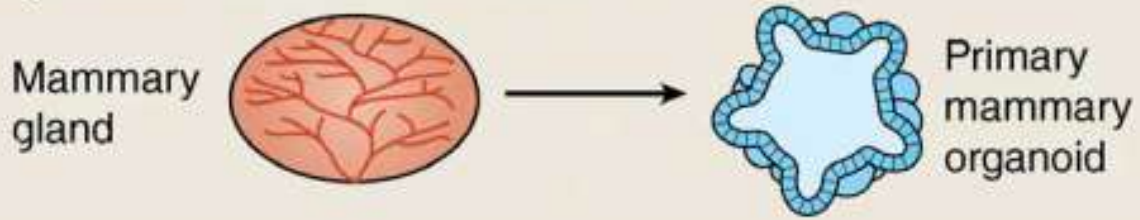


TABLE 1

Effect of different collagenase concentrations on the dissociation of the mammary tissue

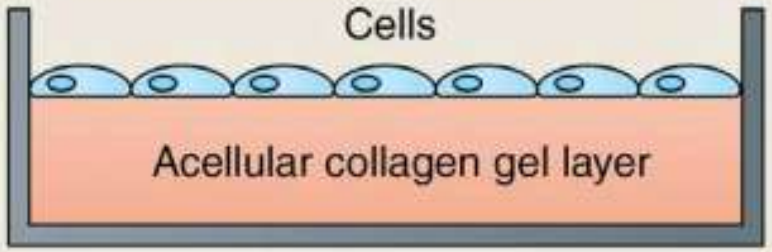
CONCENTRATION	EXPOSURE TIME	RESULTS AT 37°C.
mg/ml	*hours*	
0.025	18	Partial dissociation
0.050	3	Beginning of dissociation
0.100	2	Partial dissociation
0.200	2	Advanced dissociation

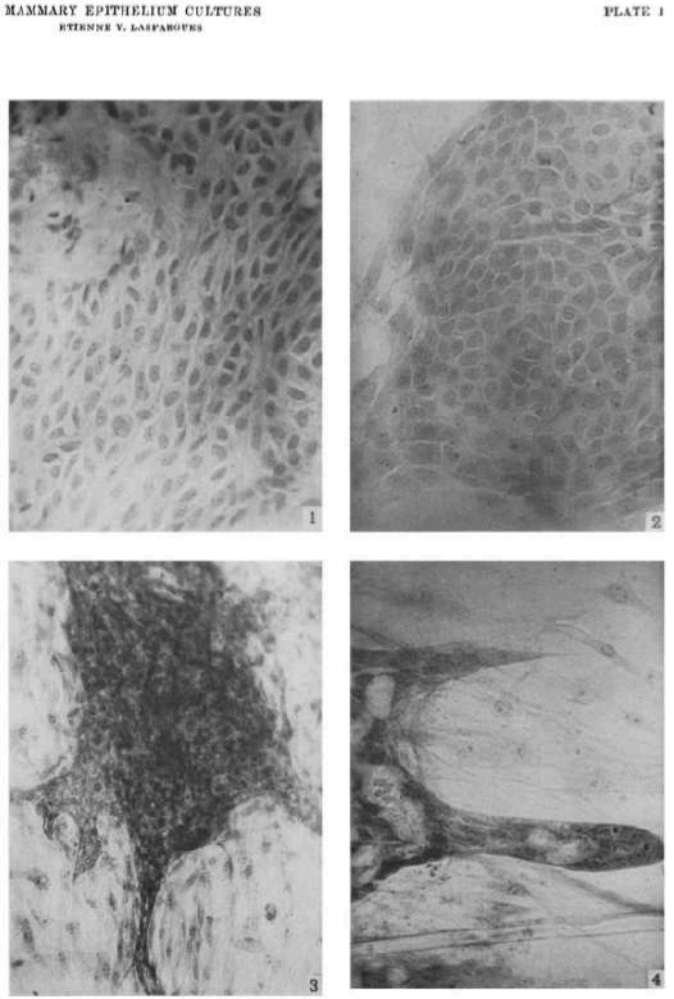
The tissue is finely minced in the collagenase solution and reduced to tiny fragments of 1 mm square or less. Simms' saline is the solvent used for the collagenase.

Lasfargues, E.Y. 1957. Anat. Rec. 127:117–129.

1960-1977

Culture of different cell types on collagen I gels





HIGH-YIELD PREPARATION OF ISOLATED RAT LIVER PARENCHYMAL CELLS

A Biochemical and Fine Structural Study

M. N. BERRY and D. S. FRIEND

From the Division of Clinical Pathology and the Department of Pathology, University of California School of Medicine, San Francisco, California 94122

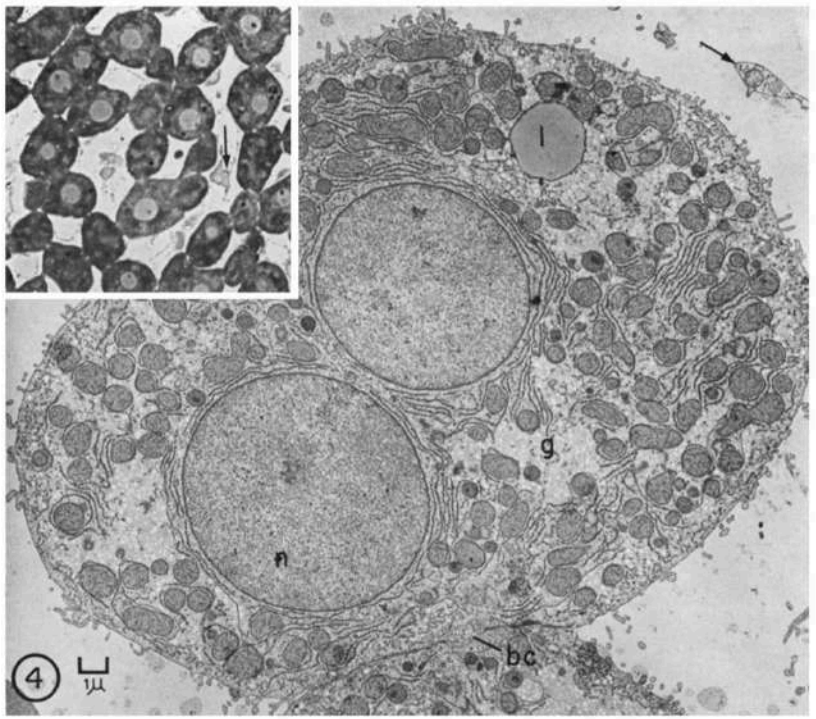


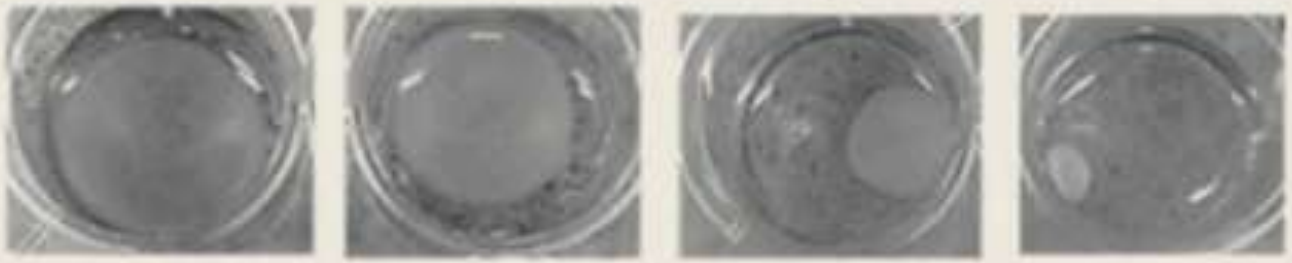
FIGURE 4 Phase and electron micrographs of liver cells during perfusion with medium containing hyaluronidase and collagenase. Adjacent hepatic parenchymal cells remain contiguous around bile canaliculi (*bc*), but endothelial (arrows) and Kupffer cells are fragmented and separated from cords of parenchymal cells. Glycogen (*g*) appears as large pools of poorly stained particles. The appearance of this binucleate liver cell is otherwise comparable to that of the normal cell before perfusion. (*l*), lipid. × 5500; inset × 1000.

Berry, M.N., and D.S. Friend. 1969. J. Cell Biol. 43:506–520.

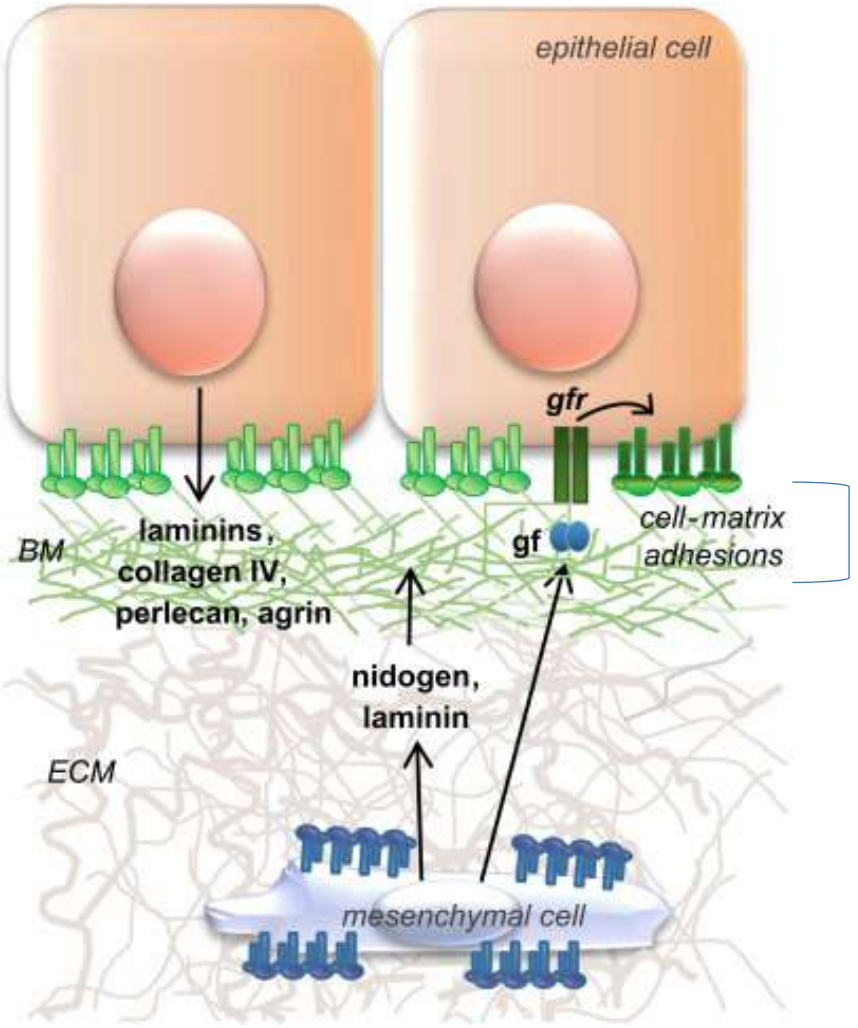
Cells on floating collagen I gels reach a higher degree of differentiation

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



1973-1979: Discovery of Fibronectin and Laminin



Basement membrane

Nelson et al. Developmental Biology, Volume 401, Issue 1, 2015, 103–109

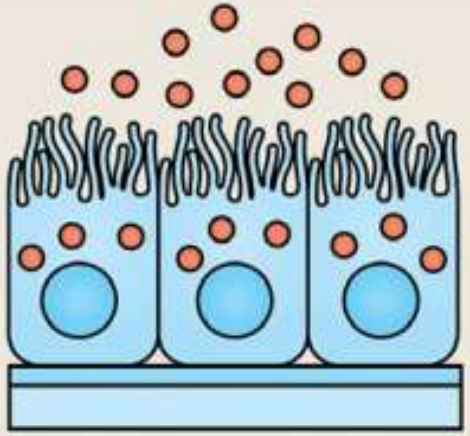
1977: Engelbreth, Holm, and Swarm isolate EHS…..that is to say Matrigel

By the 1980's…

- Were able to culture cells
- Could make collagen I gels
- Could dissagregate different tissues using collagenase
- Had characterized basemente membrane components
- EHS (Matrigel was available)

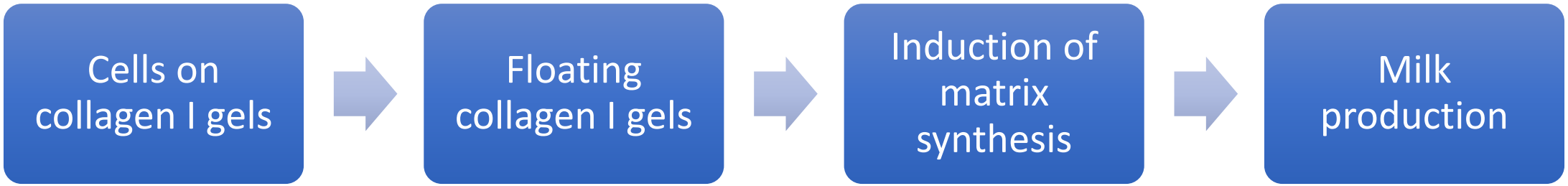
1984, 1985 Lee et al.

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



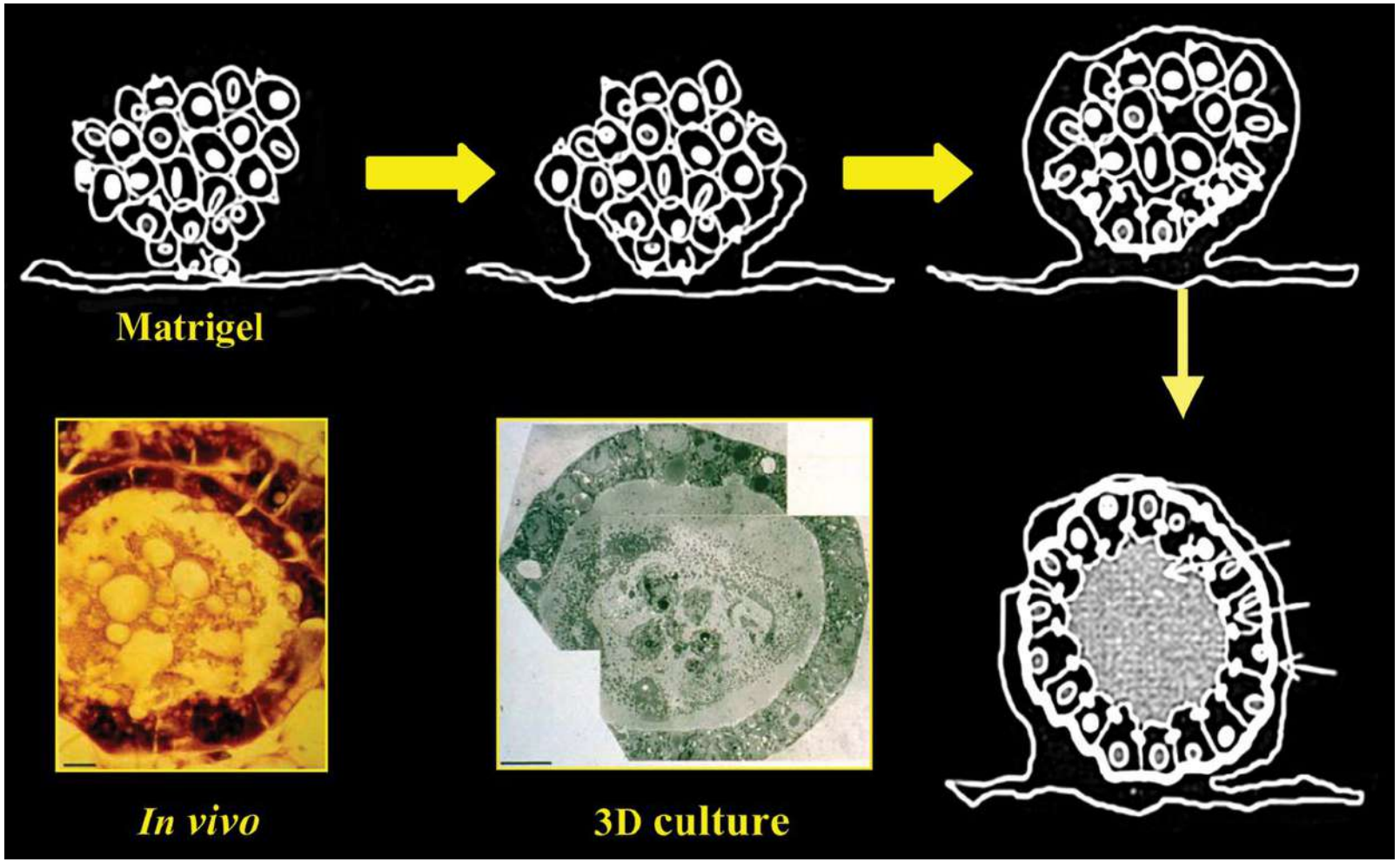
- Lee, E.Y., G. Parry, and M.J. Bissell. 1984. Modulation of secreted proteins of mouse mammary epithelial cells by the collagenous substrata. J. Cell Biol. 98:146–155..
- Lee, C.S. Kaetzel, G. Parry, and M.J. Bissell. 1985. Interaction of mouse mammary epithelial cells with collagen substrata: Regulation of casein gene expression and secretion. Proc. Natl. Acad. Sci. USA. 82:1419–1423.

Events related to milk production on floating collagen I gels



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.



M.J. Bissell Biochm. Soc. Trans. 2007;35:18-22

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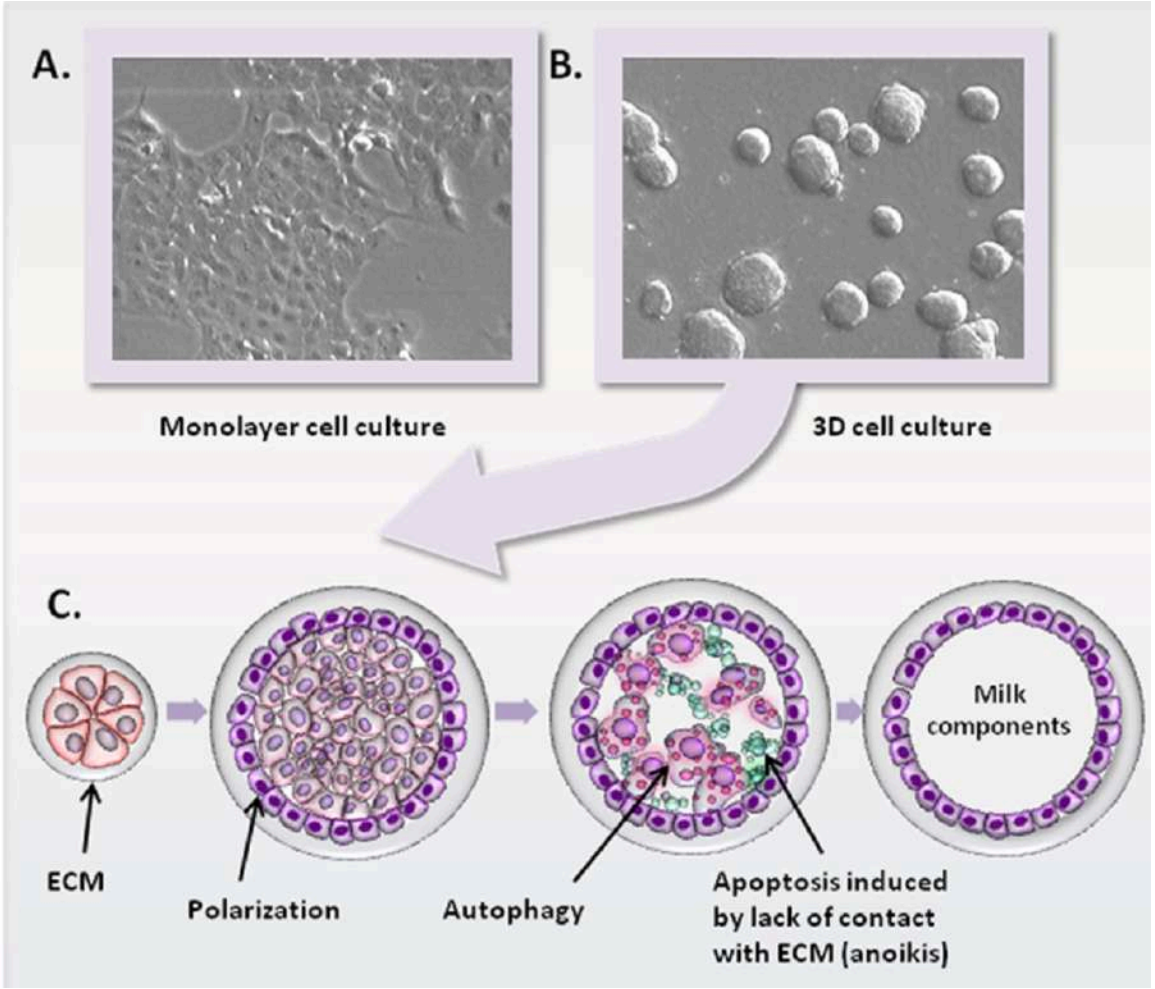
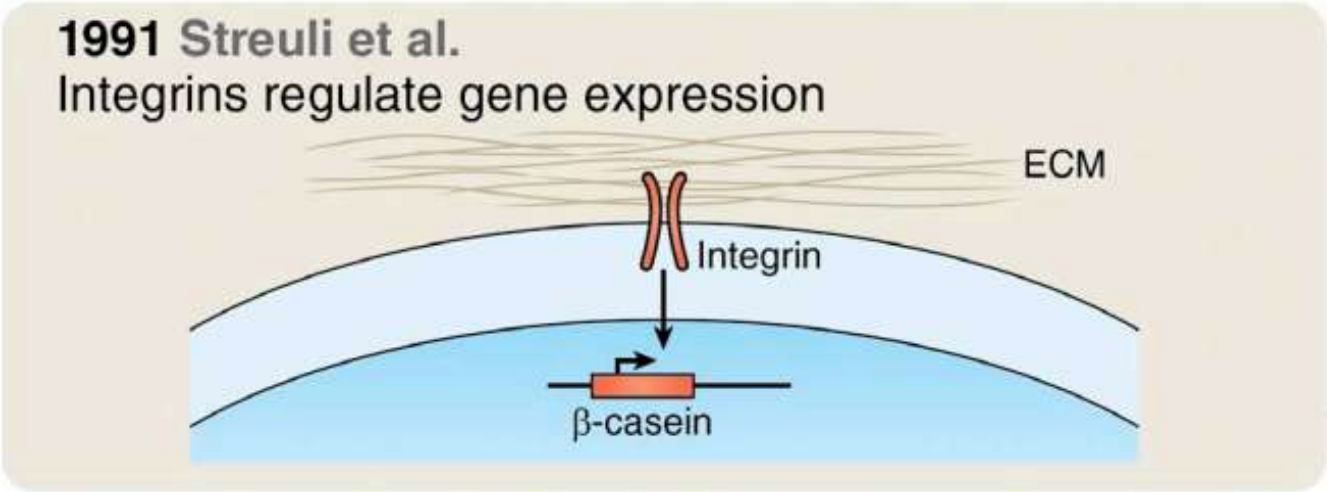


Gajewska et al. (2013). Autophagy in Development and Remodelling of Mammary ….
Dr. Yannick Bailly (Ed.), InTech, DOI: 10.5772/54558

How does the ECM govern milk production?

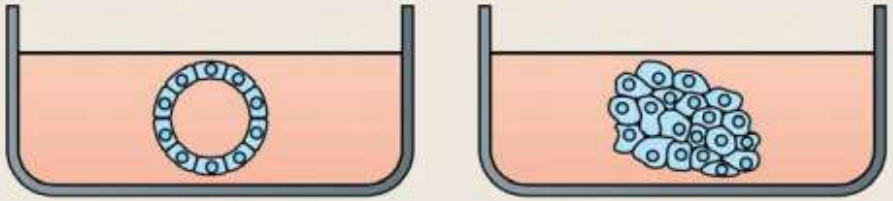


Laminin-111 through β1 and β4 integrins governs milk production

Assay to distinguish normal from malignant cells

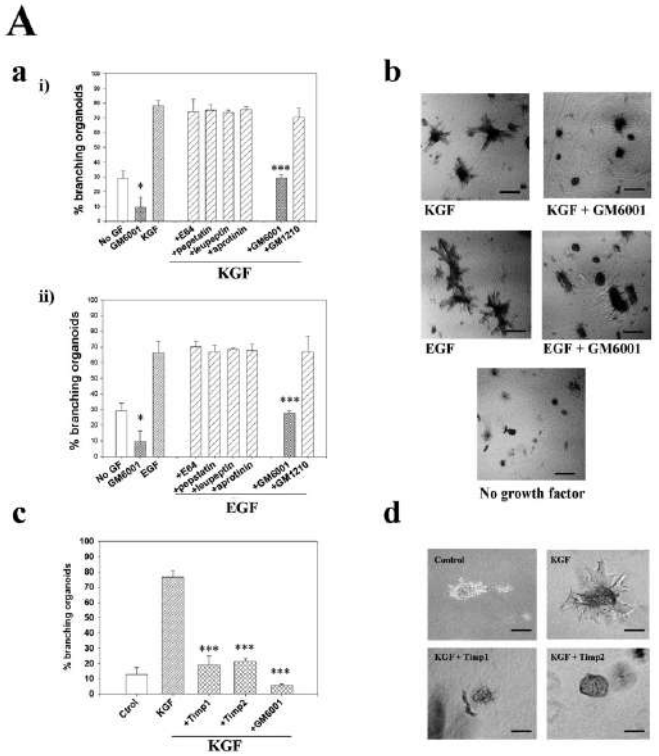
1989 Barcellos-Hoff et al. 1992 Petersen et al.

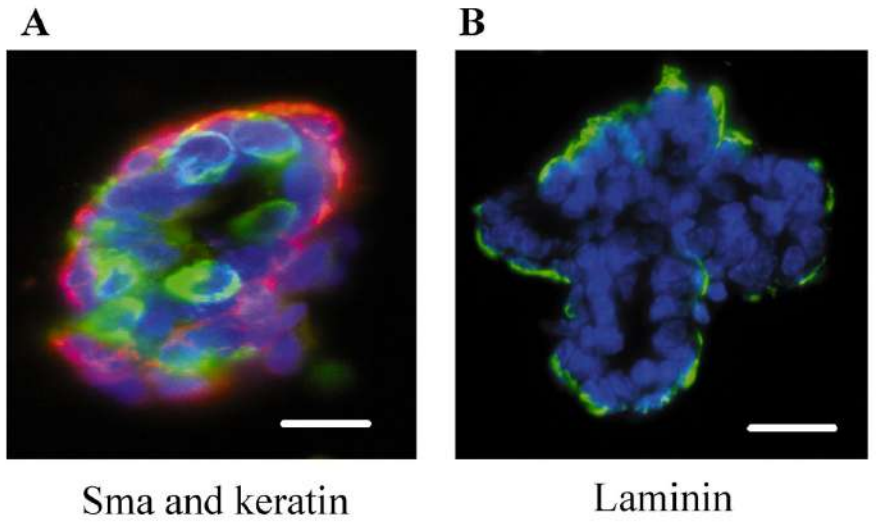
Use of a laminin-rich matrix to develop assays of mammary morphogenesis and to distinguish between healthy and malignant human epithelial cells.



Mammary epithelial cells | Breast cancer cells

1990-2000: use of 3D cultures to study morphogenesis





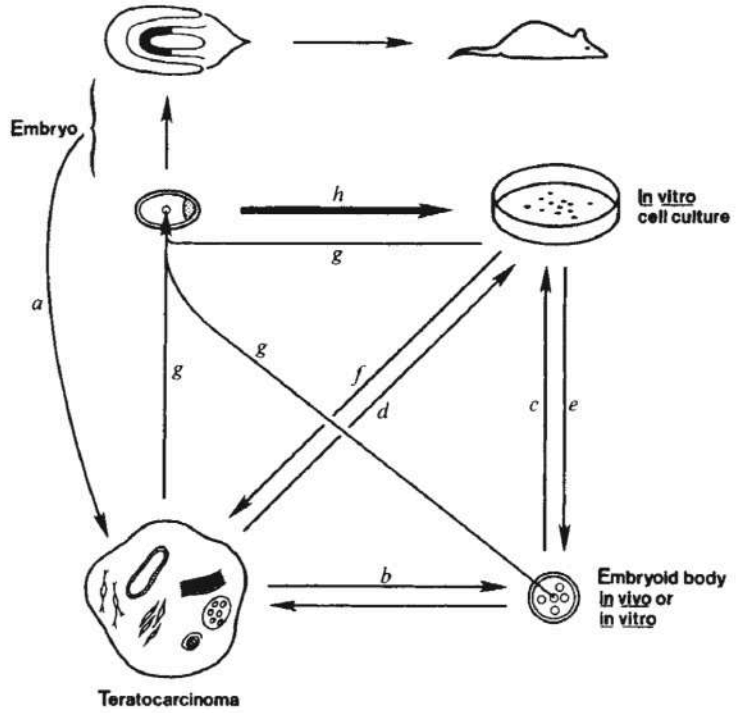
Simian et al. Development 2001;128:3117-3131

Stem cell field: 1981

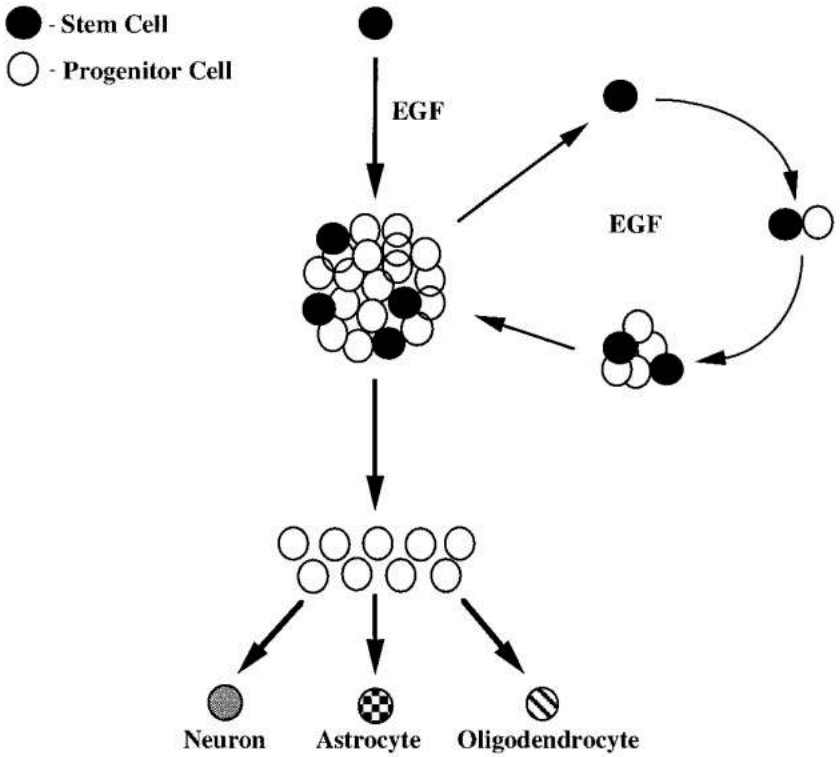
Establishment in culture of pluripotential cells from mouse embryos

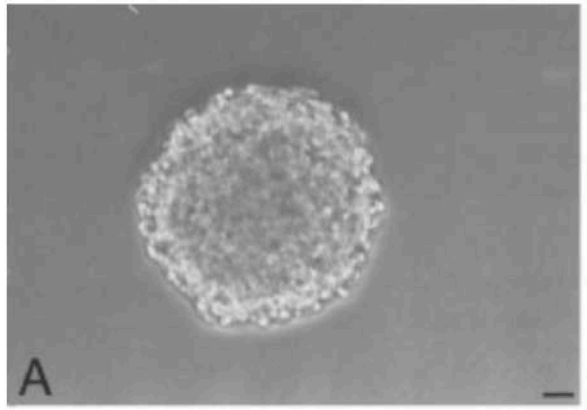
M. J. Evans* & M. H. Kaufman†

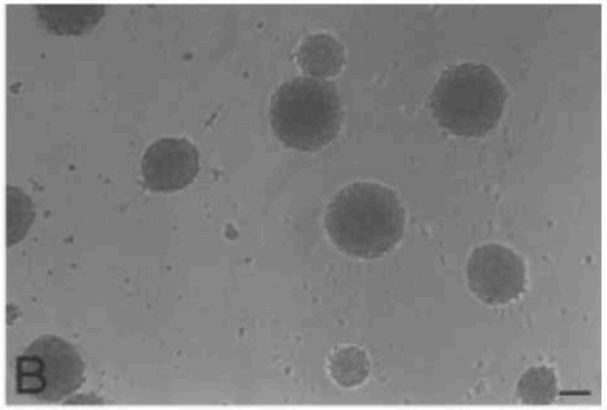
Departments of Genetics* and Anatomy†, University of Cambridge, Downing Street, Cambridge CB2 3EH, UK



1996: First neurosphere cultures

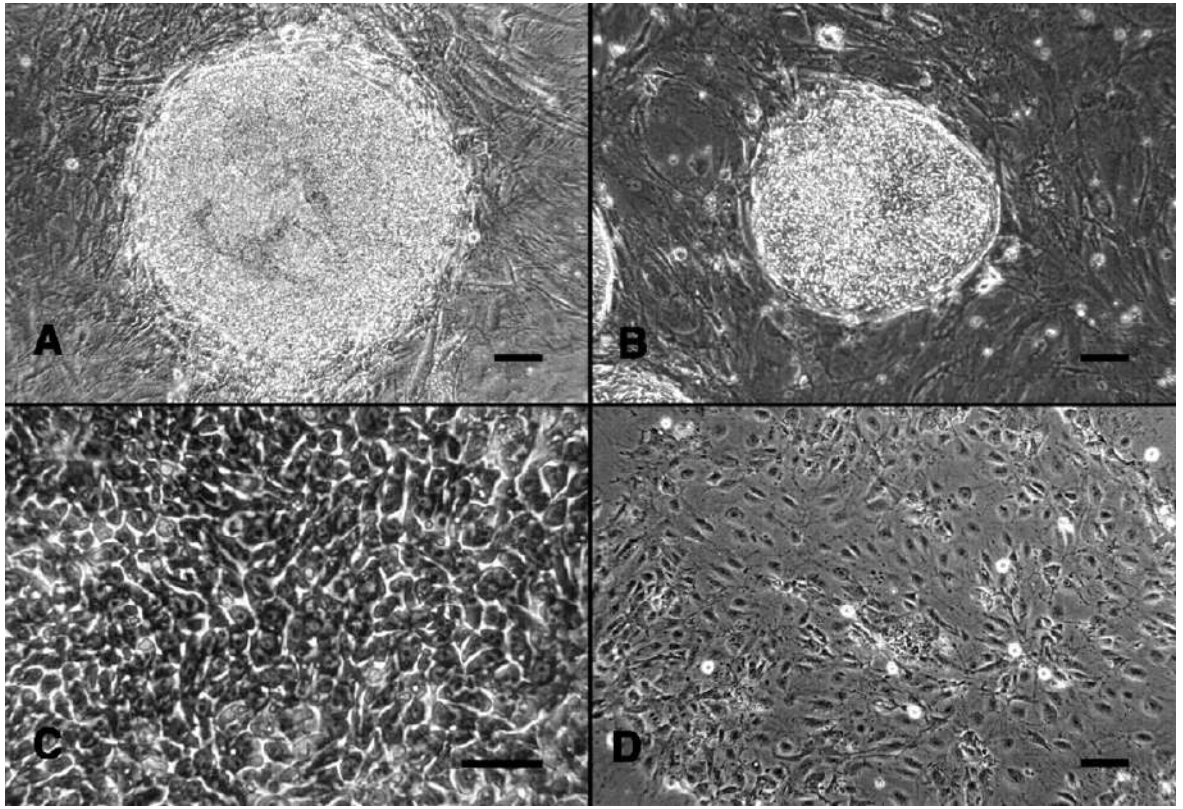






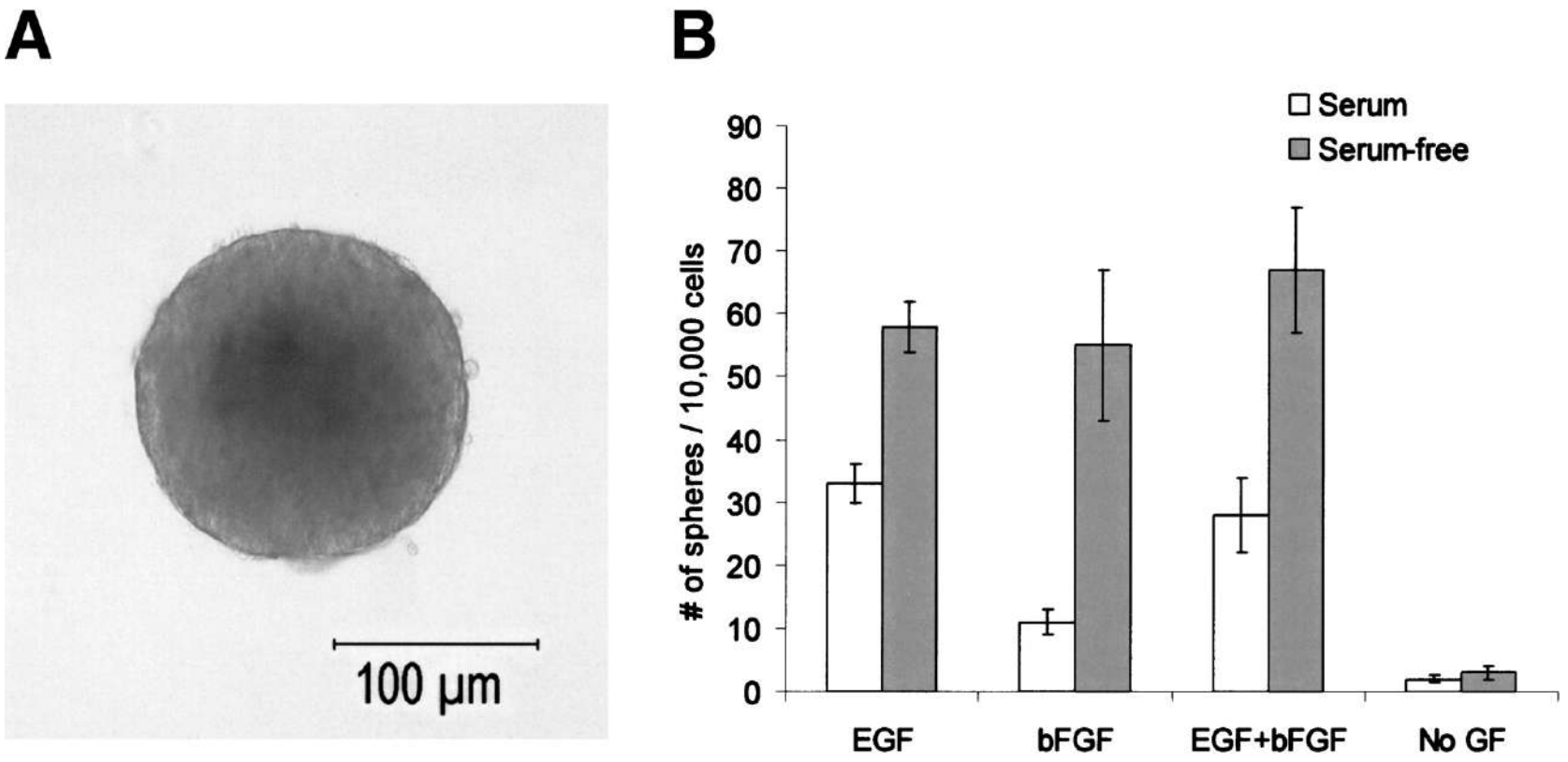
Reynolds and Weiss, 1996. Dev. Biol 175, 1-13

1998: Embryonic Stem Cell Lines Derived from Human Blastocysts

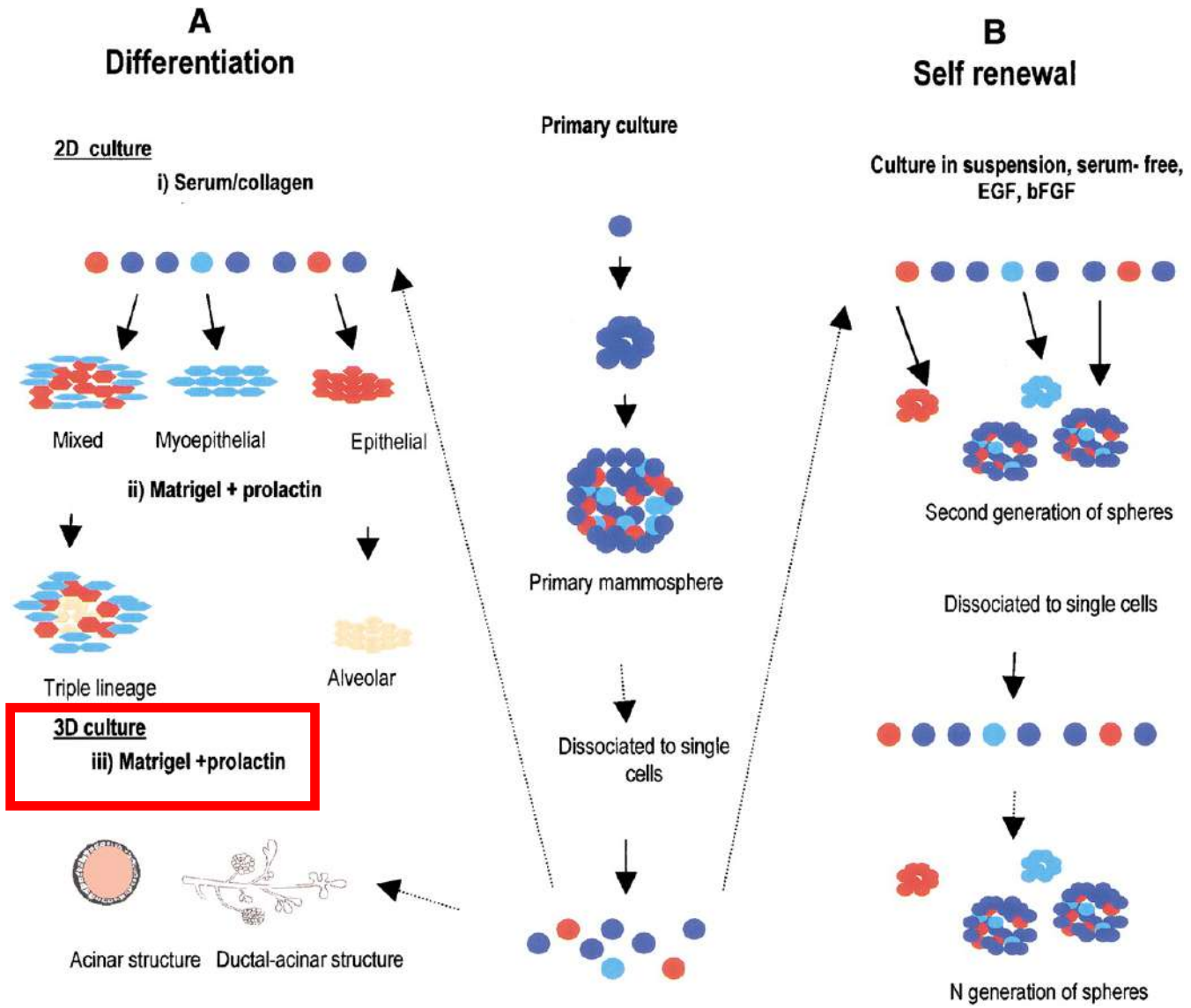


James A. Thomson et al. Science 1998;282:1145-1147

Culture of mammary gland stem cells



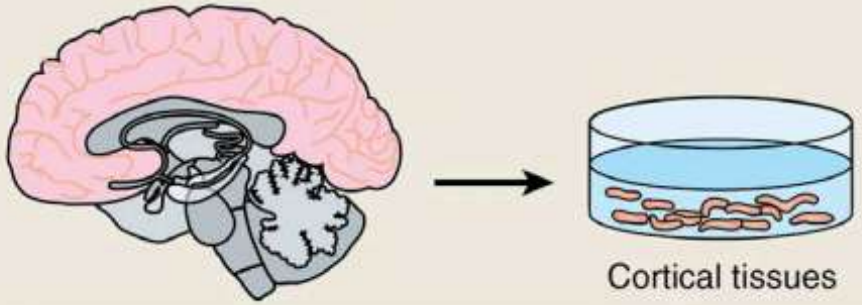
Gabriela Dontu et al. Genes Dev. 2003;17:1253-1270

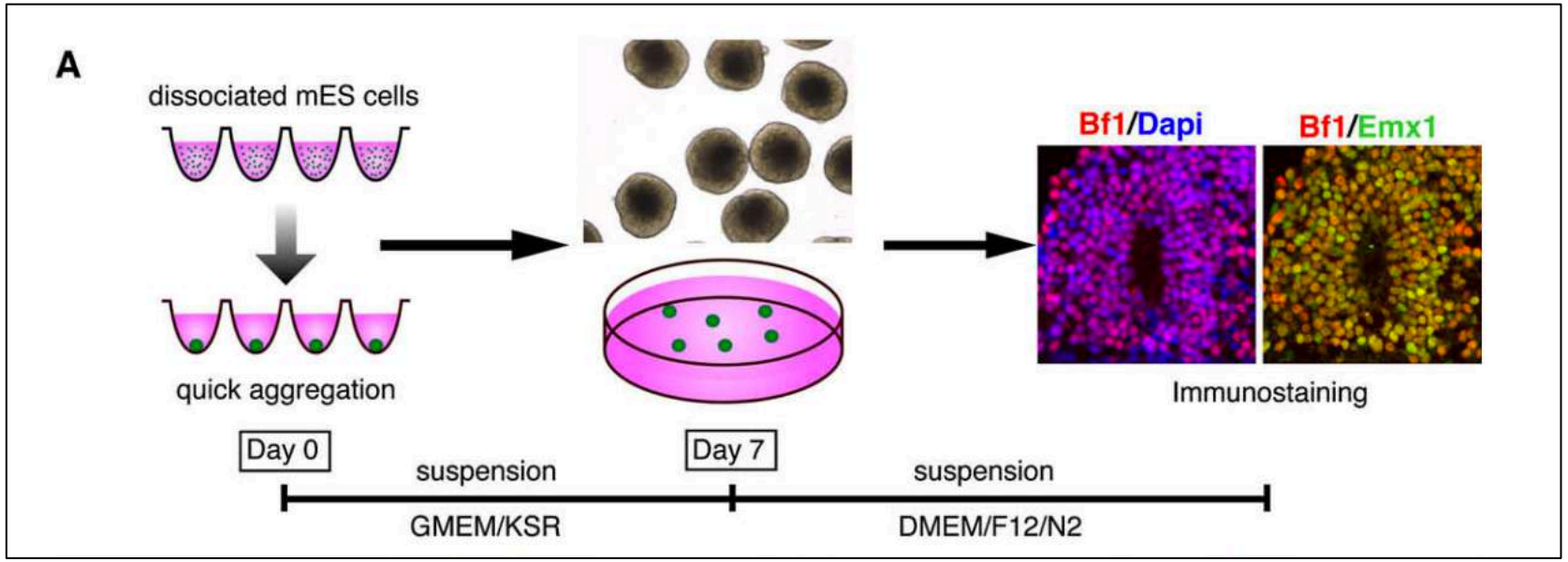


Gabriela Dontu et al. Genes Dev. 2003;17:1253-1270

2008 Eiraku et al.

Self-organized formation of polarized cortical tissues from ESCs using 3D aggregation cultures

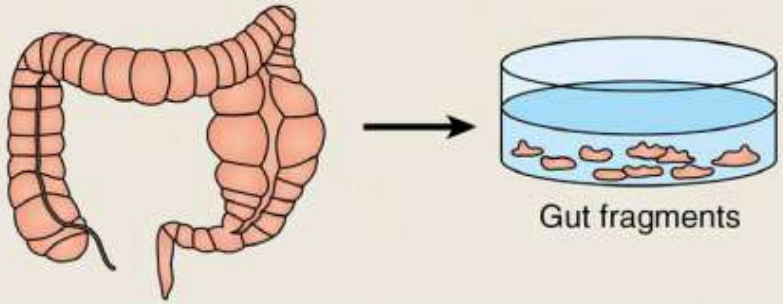


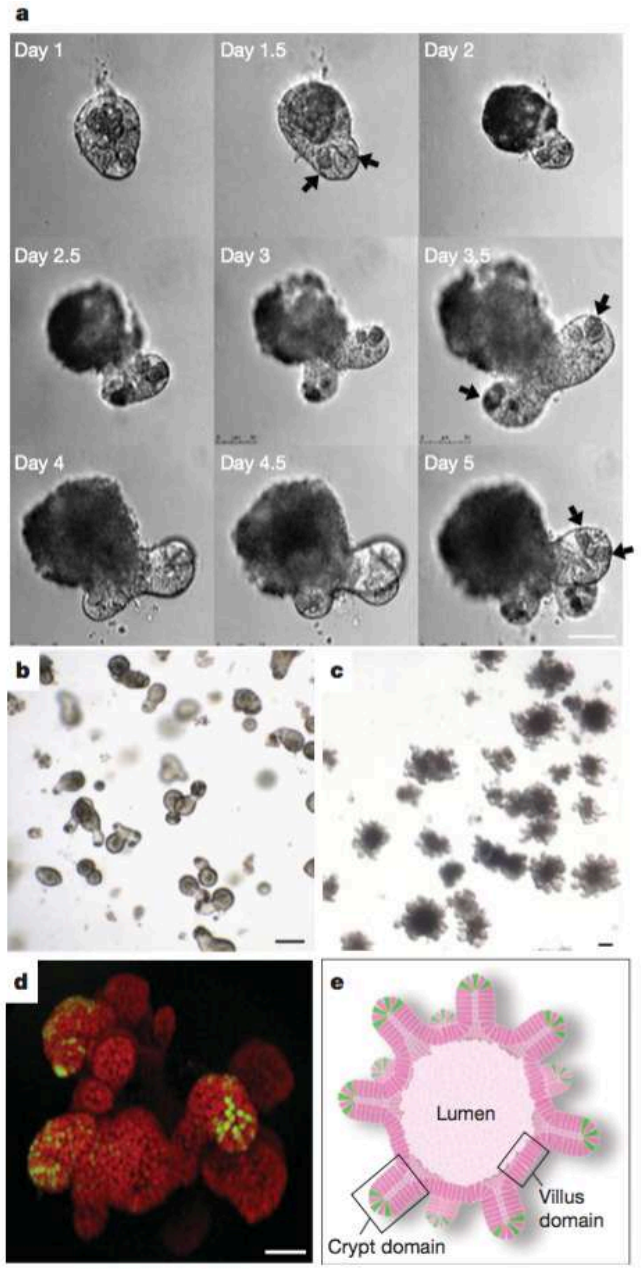


Eiraku et al. 2008. Self-organized formation of polarized cortical tissues from ESCs... Cell Stem Cell. 3:519– 532.

2009 Sato et al.

"Mini-guts": a culture system allows growth of epithelial organoids from a single Lgr5-positive stem cell

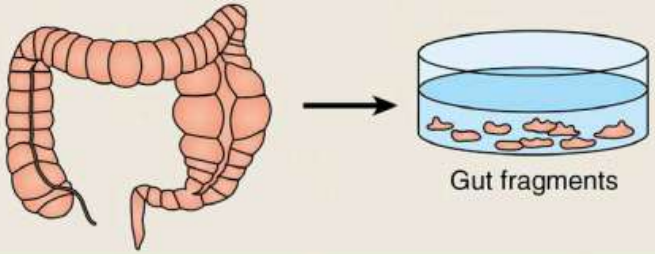




Sato et al. 2009 Single Lgr5 stem cells build crypt-villus structures in vitro... Nature. 459:262–265.

2009 Sato et al.

"Mini-guts": a culture system allows growth of epithelial organoids from a single Lgr5-positive stem cell



METHODS SUMMARY

Mice. Outbred mice 6–12 weeks old were used. Generation and genotyping of the *Lgr5–EGFP–Ires–CreERT2* allele[1] has been described previously[3]. Rosa26–lacZ or YFP–Cre reporter mice were obtained from Jackson Labs.

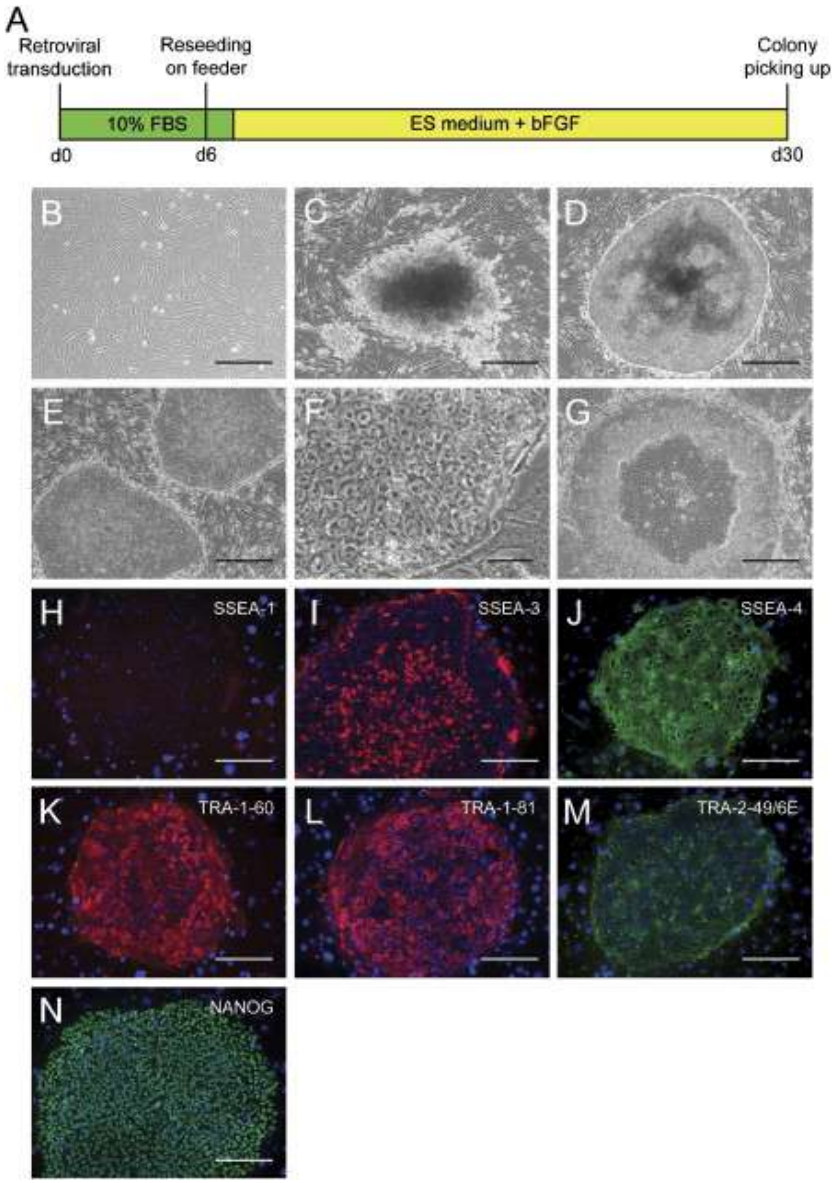
Crypt isolation, cell dissociation and cell culture. Crypts were released from murine small intestine by incubation for 30 min at 4 °C in PBS containing 2 mM EDTA (Supplementary Methods). Isolated crypts were counted and pelleted. A total of 500 crypts were mixed with 50 µl of Matrigel (BD Bioscience) and plated in 24-well plates. After polymerization of Matrigel, 500 µl of crypt culture medium (Advanced DMEM/F12 (Invitrogen)) containing growth factors (10–50 ng ml^{-1} EGF (Peprotech), 500 ng ml^{-1} R-spondin 1 (ref. 11) and 100 ng ml^{-1} Noggin (Peprotech)) was added. For sorting experiments, isolated crypts were incubated in culture medium for 45 min at 37 °C, followed by trituration with a glass pipette. Dissociated cells were passed through cell strainer with a pore size of 20 µm. GFP^{hi}, GFP^{low} and GFP^{-} cells were sorted by flow cytometry (MoFlo; Dako). Single viable epithelial cells were gated by forward scatter, side scatter and pulse-width parameter, and by negative staining for propidium iodide. Sorted cells were collected in crypt culture medium and embedded in Matrigel containing Jagged-1 peptide (1 µM; AnaSpec) at 1 cell per well (in 96-well plates, 5 µl Matrigel). Crypt culture medium (250 µl for 48-well plates, 100 µl for 96-well plates) containing Y-27632 (10 µM) was overlaid. Growth factors were added every other day and the entire medium was changed every 4 days. For passage, organoids were removed from Matrigel and mechanically dissociated into single-crypt domains, and then transferred to fresh Matrigel. Passage was performed every 1–2 weeks with a 1:5 split ratio.

The same technology was used for culture of:

- **Stomach:** Barker, N., M. Huch, P. Kujala, M. van de Wetering, H.J. Snippert, J.H. van Es, T. Sato, D.E. Stange, H. Begthel, M. van den Born, et al. 2010. Lgr5+ve stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. Cell Stem Cell. 6:25–36
- **Pancreas:** Huch, M., P. Bonfanti, S.F. Boj, T. Sato, C.J. Loomans, M. van de Wetering, M. Sojoodi, V.S. Li, J. Schuijers, A. Gracanin, et al. 2013a. Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. EMBO J. 32:2708–2721
- **Colon:** Sato, T., D.E. Stange, M. Ferrante, R.G. Vries, J.H. Van Es, S. Van den Brink, W.J. Van Houdt, A. Pronk, J. Van Gorp, P.D. Siersema, and H. Clevers. 2011. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology. 141:1762–1772.

- **Liver:** Huch, M., C. Dorrell, S.F. Boj, J.H. van Es, V.S. Li, M. van de Wetering, T. Sato, K. Hamer, N. Sasaki, M.J. Finegold, et al. 2013b. In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. Nature. 494:247–250.

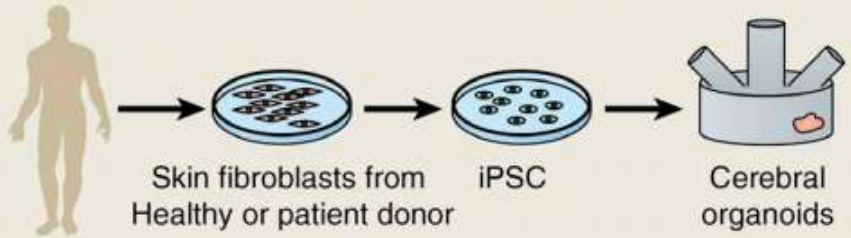
Induction of iPS cells from human dermal fibroblasts

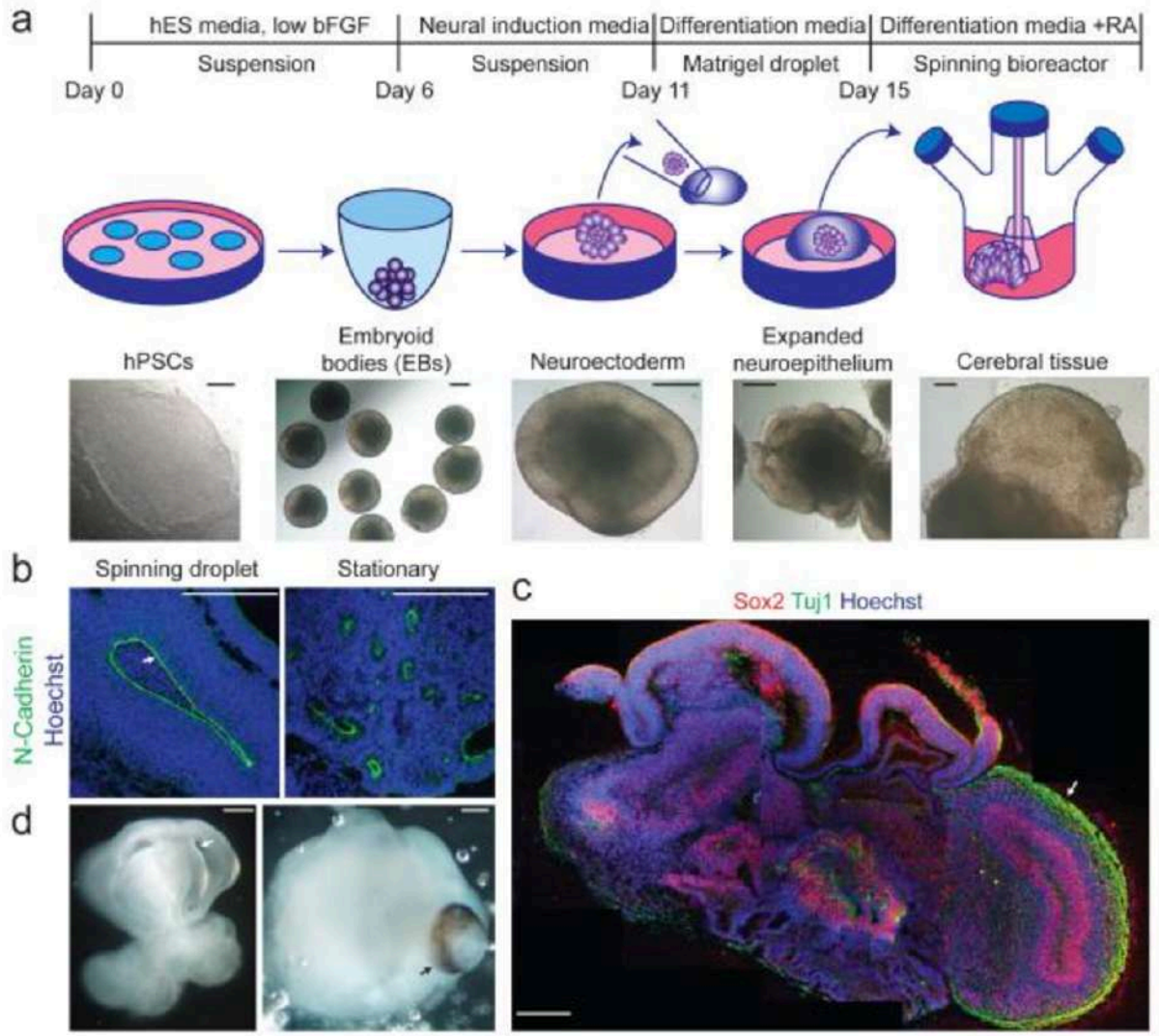


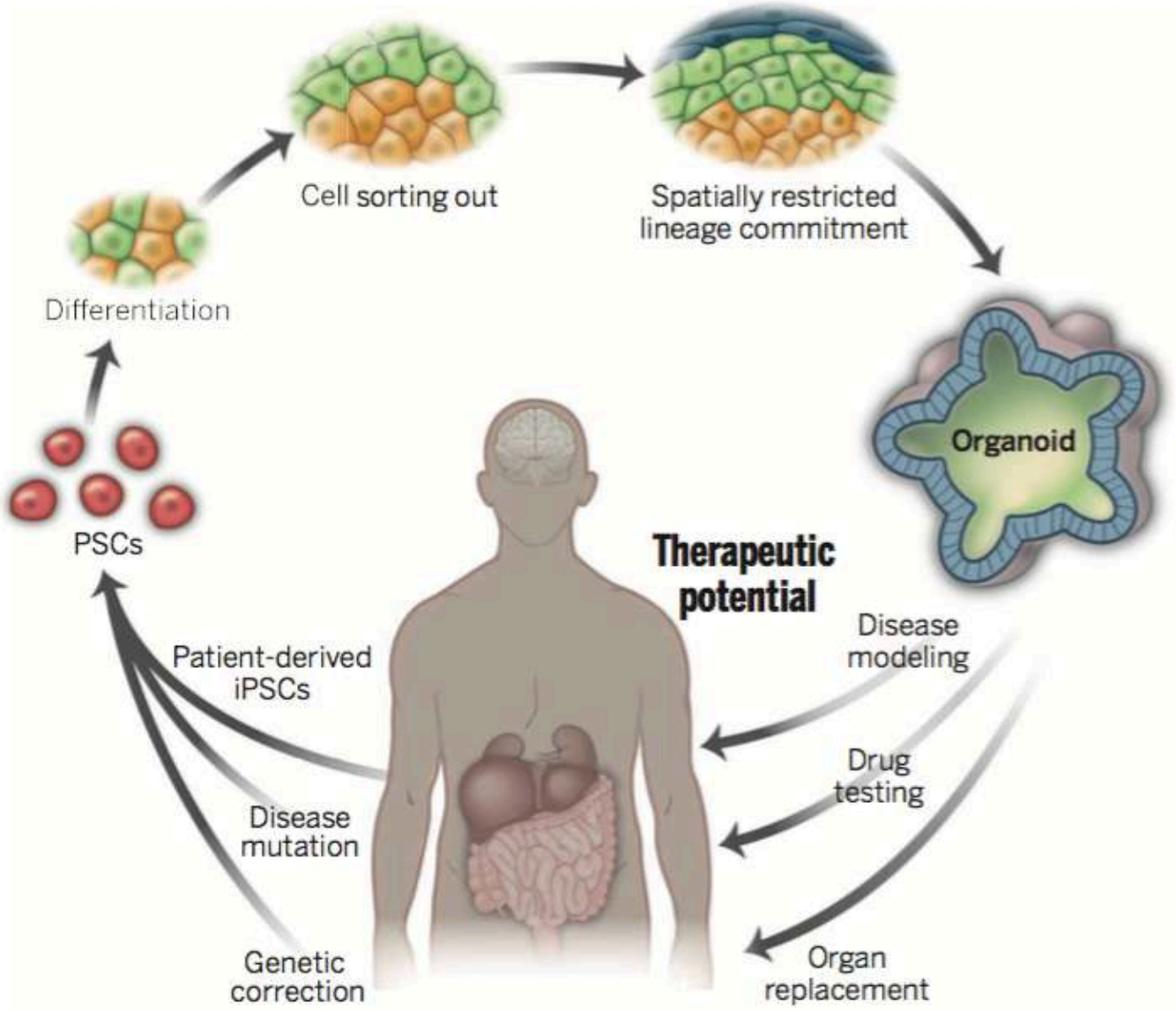
Takahashi et al 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 131:861–872.

2013 Lancaster et al.

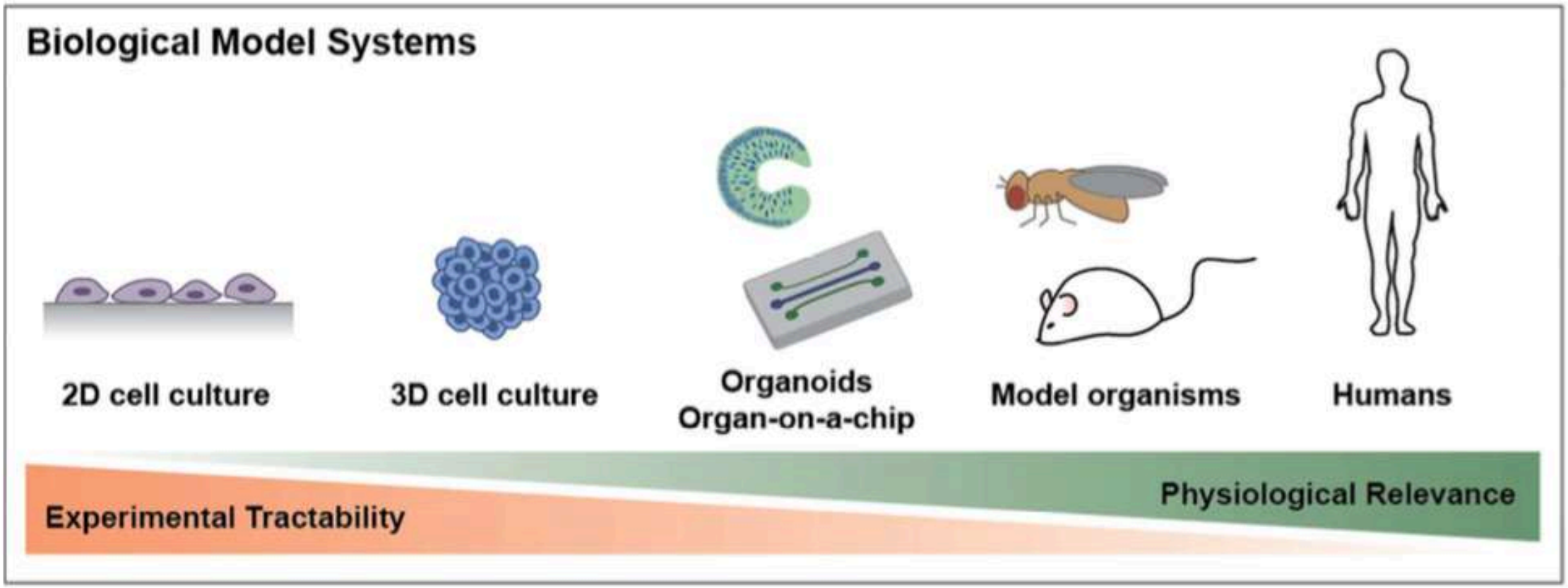
Human brain organoids are generated from iPSCs derived from cells from a patient with microcephaly.



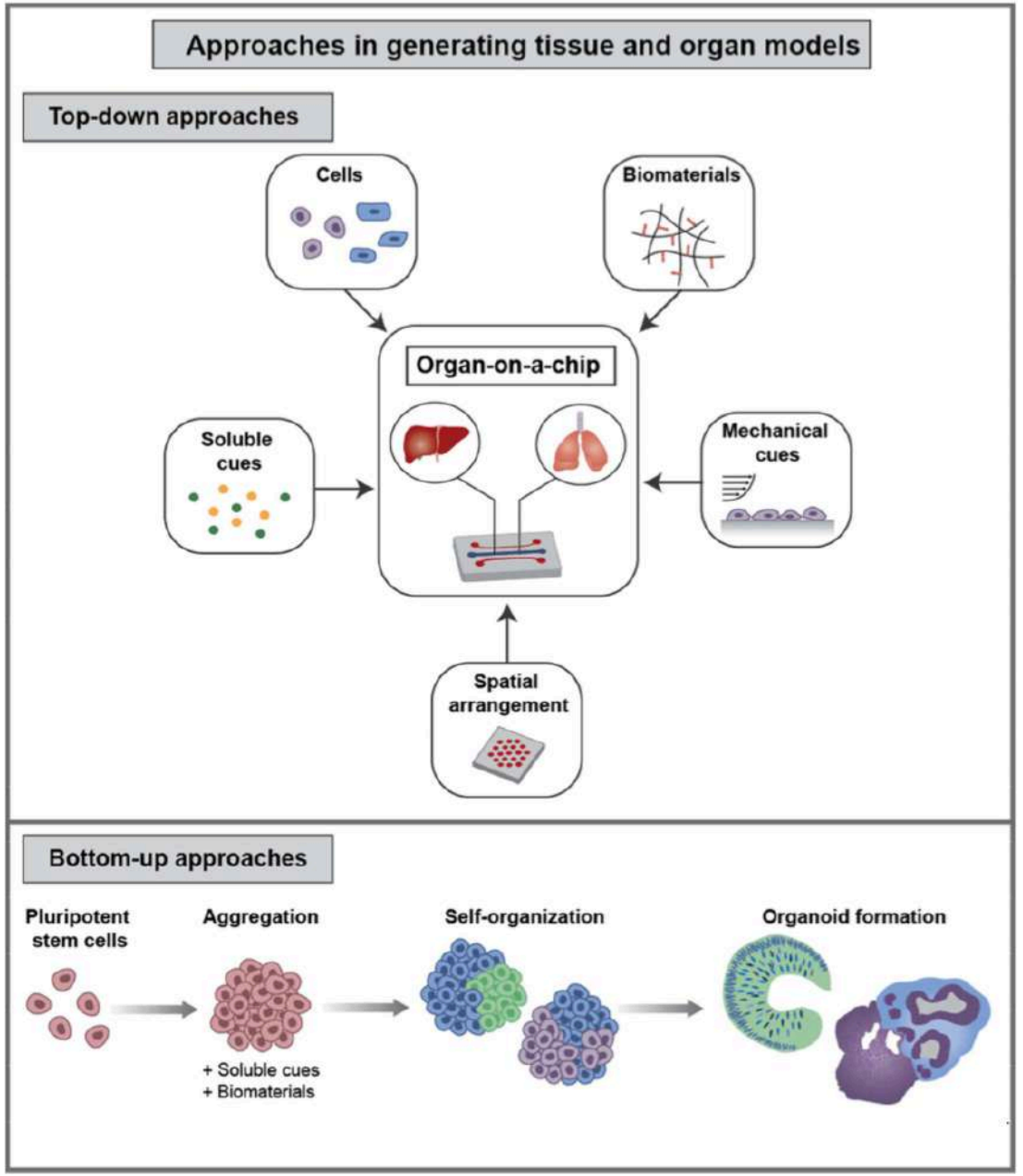




Lancaster, M.A., and J.A. Knoblich. 2014. Organogenesis in a dish: Modeling development and disease using organoid technologies. Science. 345:1247125.

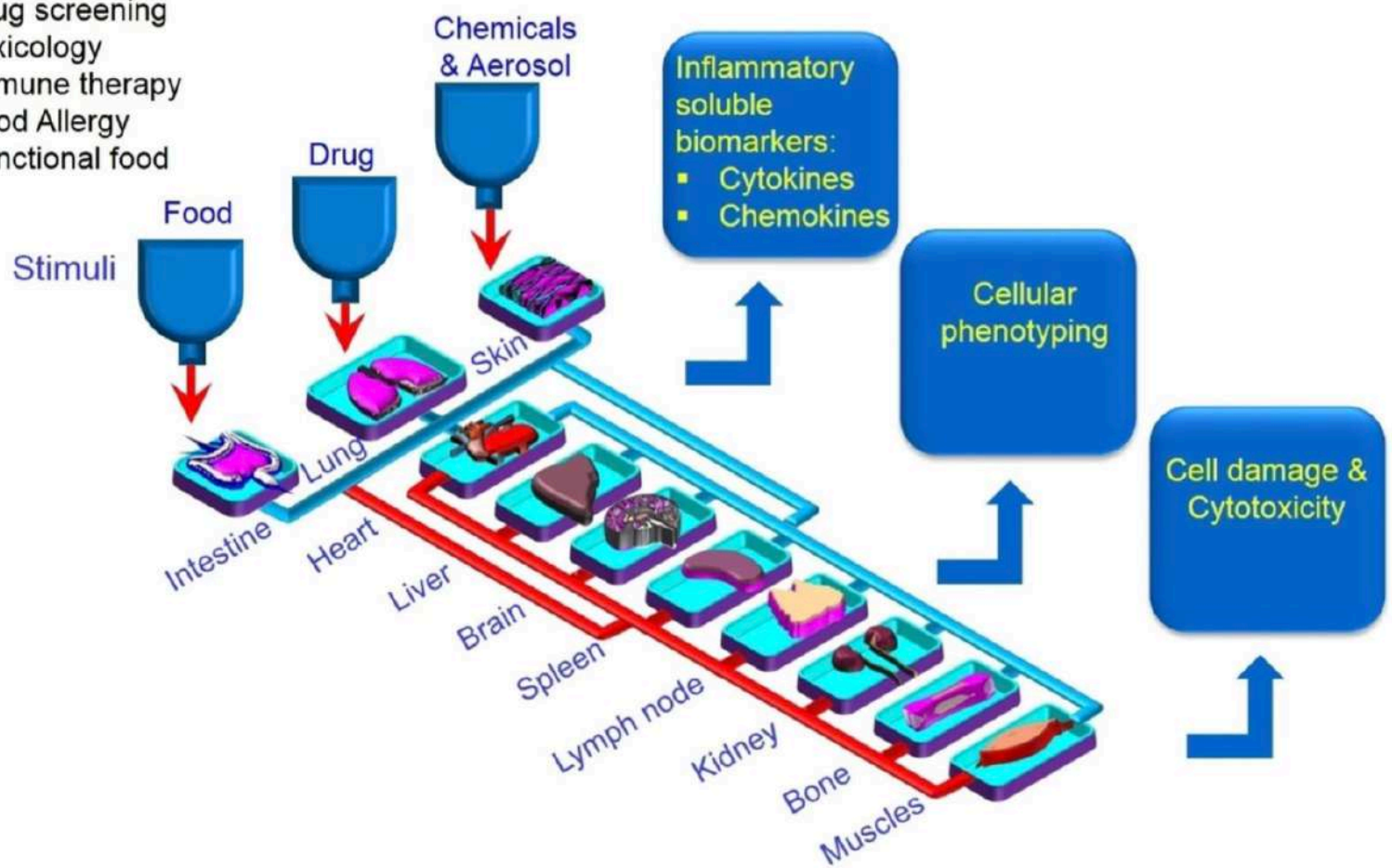


Jackson & Lu, 2016. Integrative Biology, 13;8(6):672-83



Jackson & Lu, 2016. Integrative Biology, 13;8(6):672-83

- Disease modeling
- Drug screening
- Toxicology
- Immune therapy
- Food Allergy
- Functional food



Ramadan and Gijs, 2014. Lab Chip. 2015 Feb 7;15(3):614-36

Thanks for listening & cheers!

Me

Mina J. Bissell

Funding

- Marina Simian: Instituto Nacional del Cáncer, Ministerio de Salud de la Nación, Argentina.
- Mina J. Bissell: National Cancer Institute, the U.S. Department of Energy, the U.S. Department of Defense, and the Breast Cancer Research Foundation