Microphysiological Models: Tools and Applications for Developmental Toxicology

Sid Hunter

Biomolecular and Computational Toxicology Division

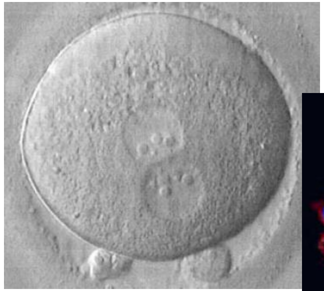
Center for Computational Toxicology and Exposure

ORD/ US EPA

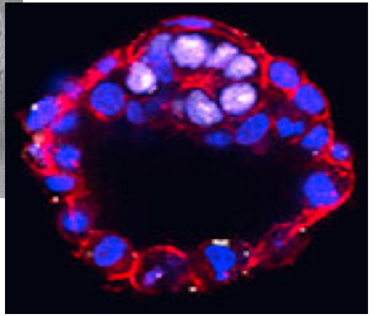
Disclaimer: The views and opinions in this presentation are those of the presenter and does not represent US EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Sid Hunter has no financial conflict of interest

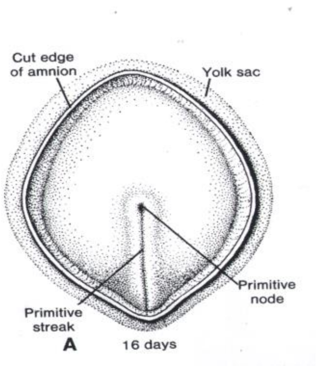
From the time of fertilization through organogenesis the conceptus undergoes profound structural and functional changes



Ebner et al., Figure 1B



Bedzhov and Zernicka-Goetz, Figure 3A



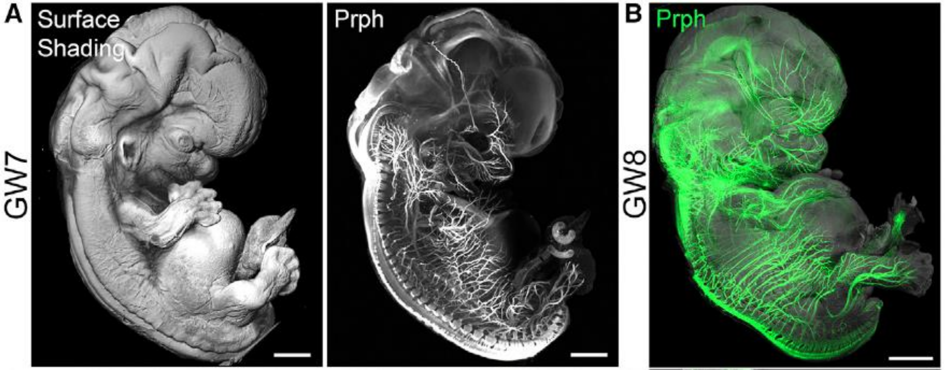
Sadler, Medical Embryology, Figure 5-1

Hunter. Original

Hypothesis: Understanding the causes and prevention of birth defects requires advancing models for human biology-based assessments and decisions

Understanding the complex genesis of human birth defects will require models that recapitulate the physiological environment of pregnancy, mimic chemical exposure and the consequences of those exposures on developing cells, tissues and organs.

http://www.cdc.gov/ncbddd/birthdefects/index.html



Belle et al., 2017, Cell 169, 161-173

Microphysiological System (MPS): cell culture systems replicating (patho)physiology through engineered organ architecture and functionality. This includes especially 3D-(co-)cultures such as organoids, organ-on-chip models, and multi-organ models, as well as the technologies to engineer and analyze these systems.

MICROPHYSIOLOGICAL SYSTEMS WORLD SUMMIT
May 30th – June 3rd, 2022

https://mpsworldsummit.com

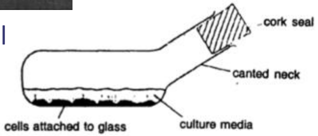
Incredible Advancements in the Tools and Approaches to Cell Culture

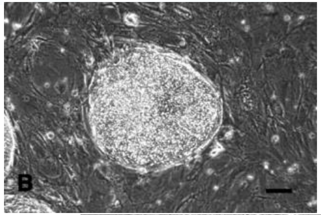
Wilhelm Roux

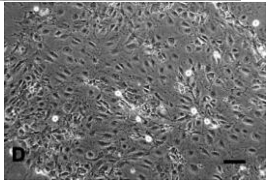
1885 Chick medullary plate

Alexis Carrel

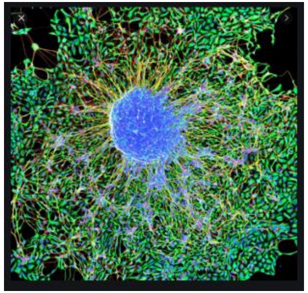
1923







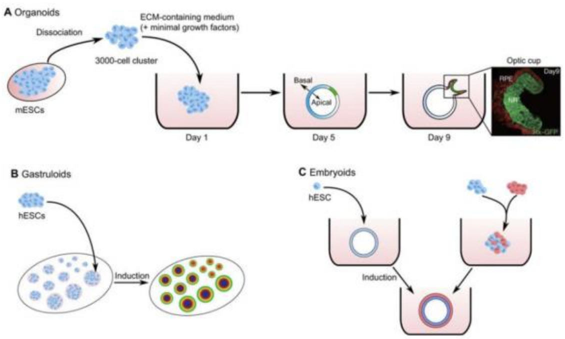
Thompson et al., *Science* 06 Nov 1998: Vol. 282, Issue 5391, pp. 1145-1147



https://www.the-scientist.com/image-of-the-day/image-of-the-day-monkeying-around-34841

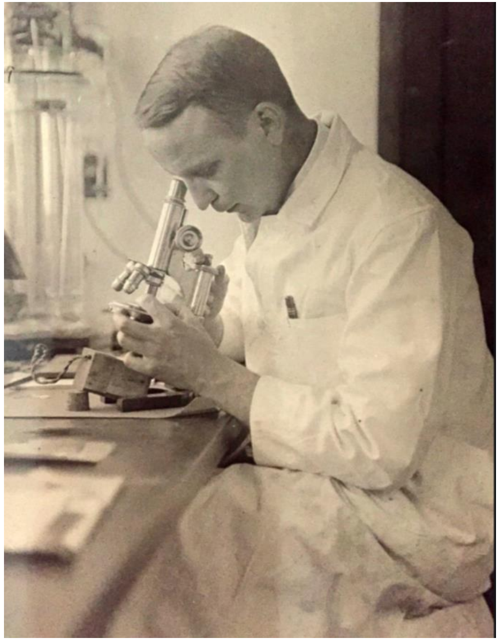
UNIVERSITY OF WISCONSIN-MADISON, [S.C. VERMILYEA, S. GUTHRIE, T.G. GOLOS, M.E. EMBORG]

Self-organization into organoids, gastruloids and embryoids.

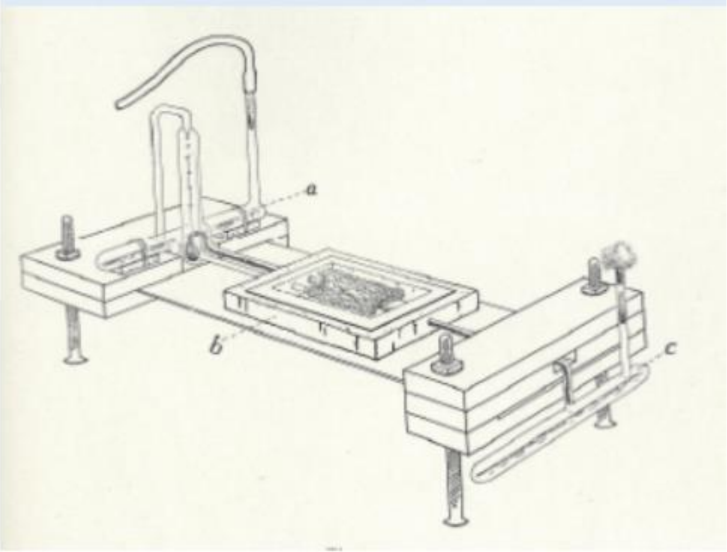


Mijo Simunovic, and Ali H. Brivanlou Development 2017;144:976-985

Development of continuous flow hanging drop culture device - 1912



Montrose T Burrows



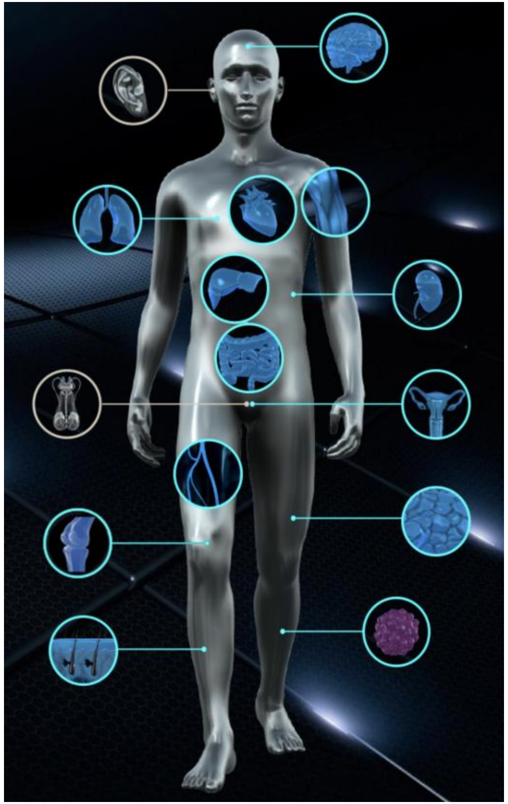
A method of furnishing a continuous supply of new medium to a tissue culture In Vitro†

Montrose T. Burrows

First published:March 1912 | https://doi.org/10.1002/ar.1090060307 | Citations: 21

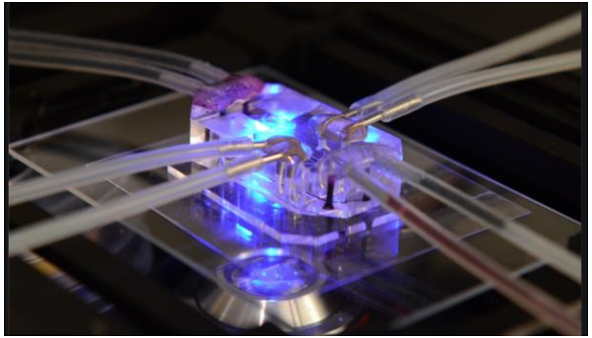
† Read before the American Association of Anatomists, December 27, 1911, at Princeton, N. J.

Organ-on-Chip, Microphysiological Models are being developed for many organs



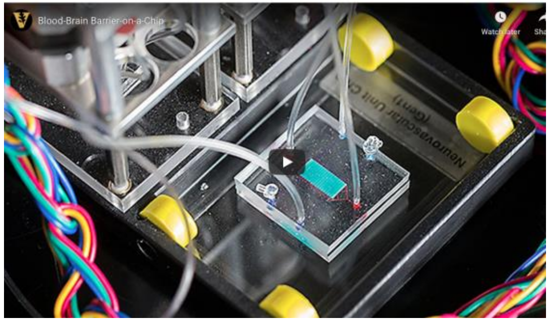
https://ncats.nih.gov/tissuechip/chip

Lung on a Chip



wyss.harvard.edu

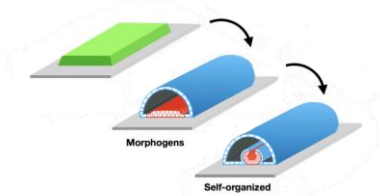
NeuroVascular Unit Chip



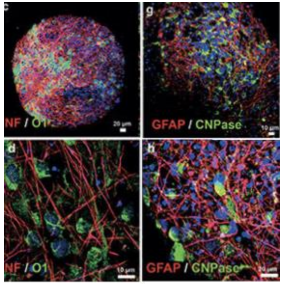
https://news.vanderbilt.edu/2016/12/06/blood-brain-barrier-on-a-chip-sheds-new-light-on-silent-killer/

Many MPS models can be used to assess developmental toxicity

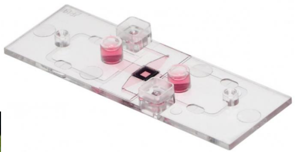
Computational Models
Stem Cell Models
Engineered Tissue Models
Self Organization in Organoids, Gastruloids and Embryoids
Chip-Based Models



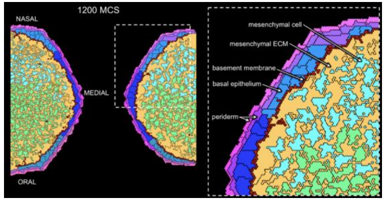
Karzbrun et al., 2021



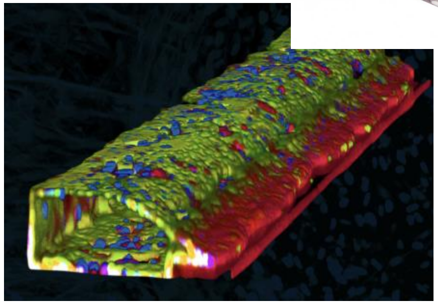
Pamies et al., 2017



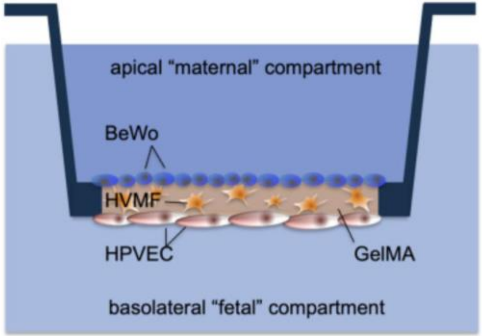
https://ibidi.com



Hutson et al., 2017



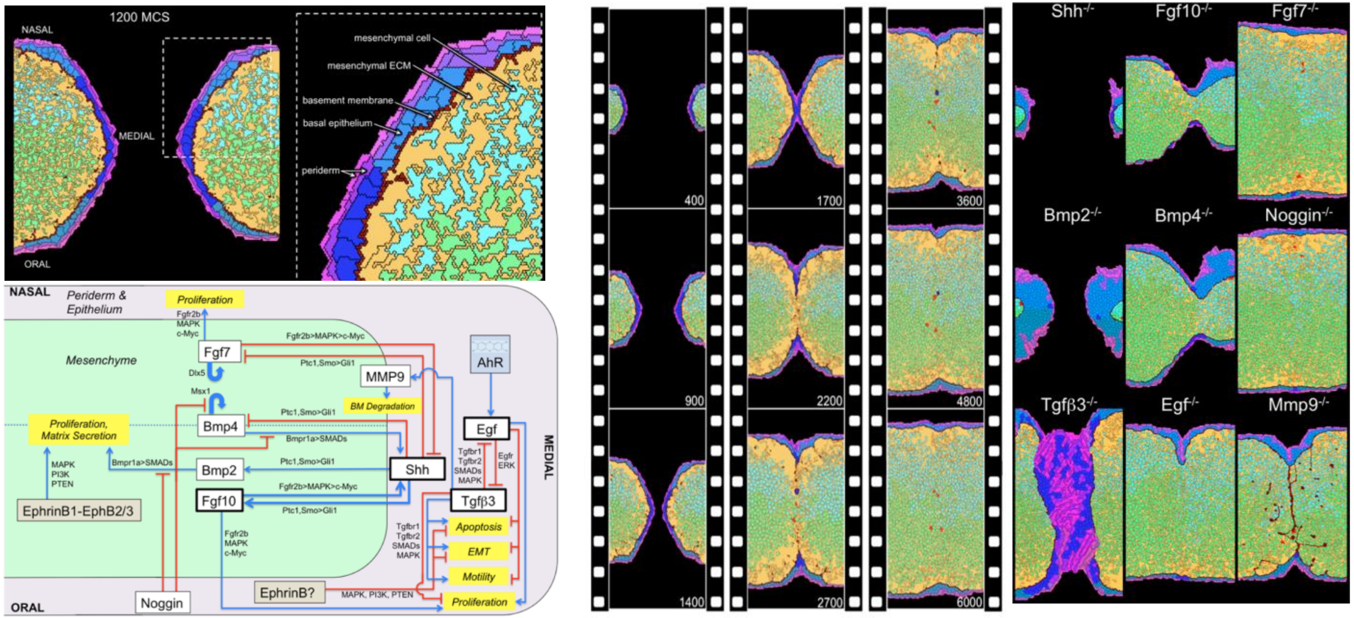
https://www.mimetas.com



Kreuder et al., 2020

Computational Models

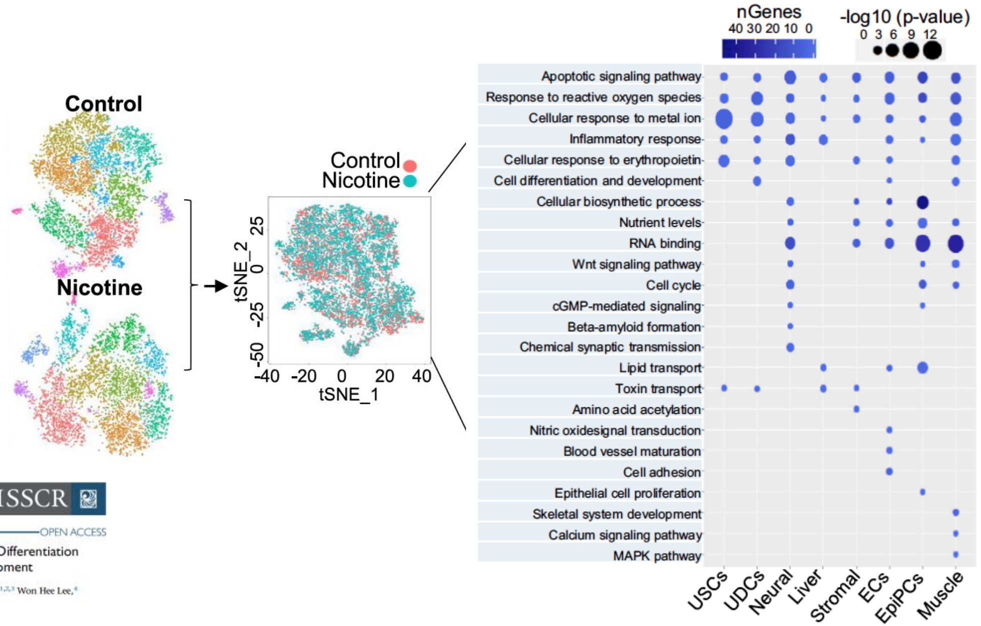
Models based upon cellular and molecular relationships driving cellular responses
Tissue models – Limb, palate, neurovascular unit, vasculogenesis, somite formation
Enables hypothesis building and testing. Cybermorphs



https://pubs.acs.org/doi/pdf/10.1021/acs.chemrestox.6b00350

Pluripotent Stem Cell Models

Focus on differentiation, cytotoxicity, proliferation
Directly plated, embryoid body, spheroid culture models
Directed or not-directed differentiation
Endpoints: Morphological, metabolomic, differentiation-marker expression
Endpoints: Single feature, many features, single cell approach



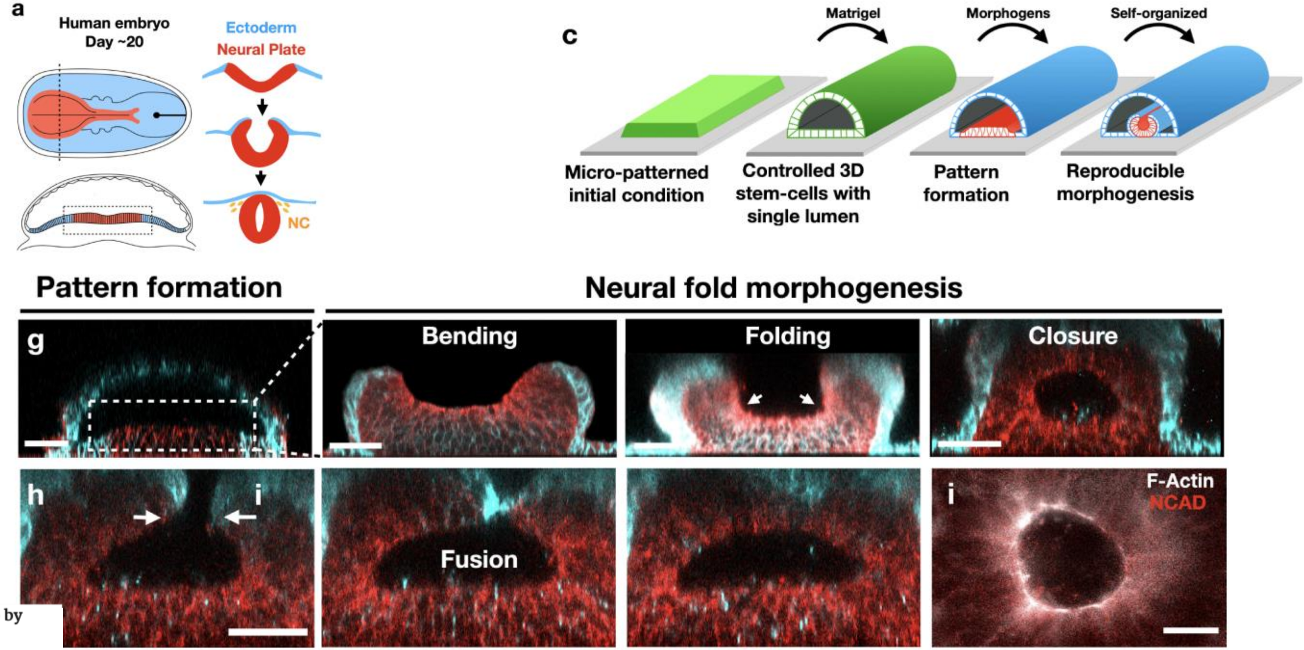
Stem Cell Reports
Article
ISSCR
OPEN ACCESS

Single-Cell RNA Sequencing of Human Embryonic Stem Cell Differentiation Delineates Adverse Effects of Nicotine on Embryonic Development

Hongchao Guo,[1,2,3,5] Lei Tian,[1,2,3,5] Joe Z. Zhang,[1,2,3] Tomoya Kitani,[1,2,3] David T. Paik,[1,2,3] Won Hee Lee,[4] and Joseph C. Wu[1,2,3,*]
[1]Stanford Cardiovascular Institute, 265 Campus Drive G1120B, Stanford, CA 94305, USA
[2]Institute for Stem Cell Biology and Regenerative Medicine, Stanford, CA 94305, USA
[3]Division of Cardiology, Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA
[4]Department of Basic Medical Sciences, University of Arizona College of Medicine, Phoenix, AZ 85004, USA

Engineered Models

Morphological Models of Tissues or Processes
Embryoid Bodies, Bioprinted, Geometrically Constrained



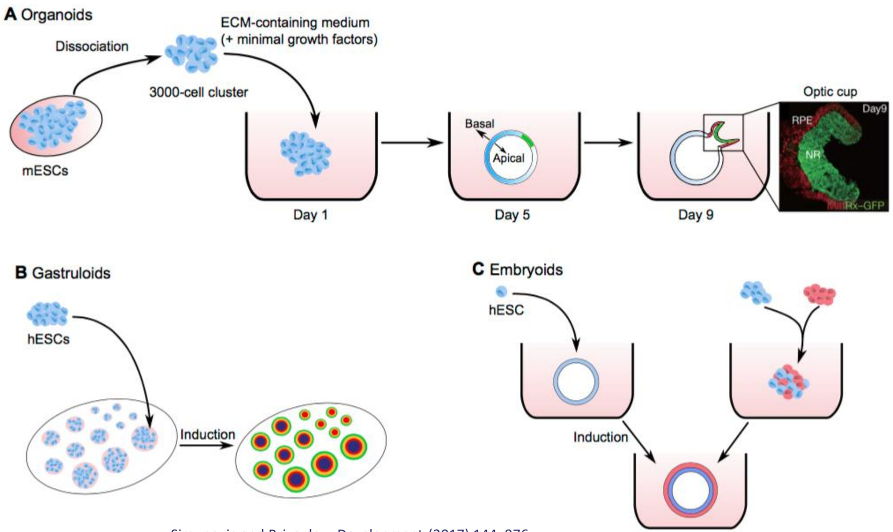
> Nature. 2021 Nov;599(7884):268-272.

Human neural tube morphogenesis in vitro by geometric constraints

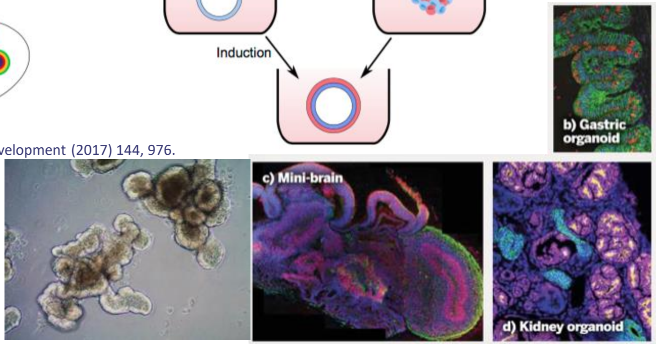
Eyal Karzbrun [#, 1, 2], Aimal H Khankhel [#, 3], Heitor C Megale [4], Stella M K Glasauer [5, 6], Yofiel Wyle [5], George Britton [7], Aryeh Warmflash [8, 9], Kenneth S Kosik [5, 6], Eric D Siggia [10], Boris I Shraiman [4, 11], Sebastian J Streichan [12, 13]

PMID: 34707290 PMCID: PMC8828633 DOI: 10.1038/s41586-021-04026-9

Self Organization in Organoids, Gastruloids and Embryoids Models



Simunovic and Brivanlou, Development (2017) 144, 976.



https://www.rsb.org.uk/biologist-features/from-organoids-to-gastruloids

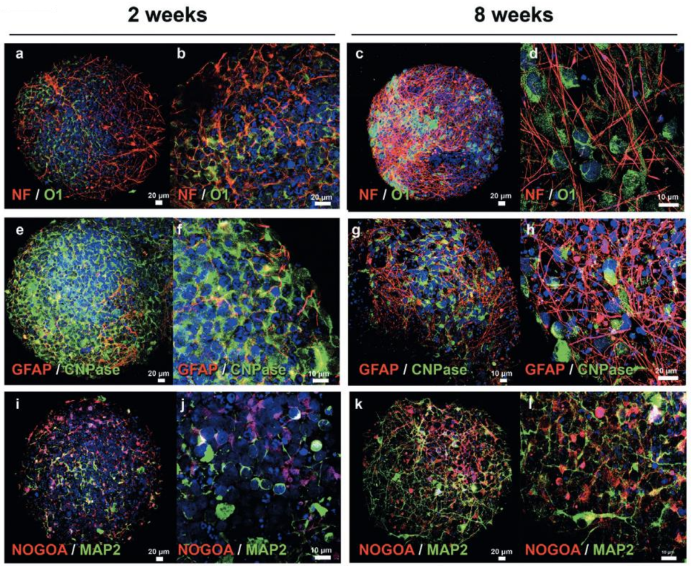
Review > Development. 2017 Mar 15;144(6):976-985. doi: 10.1242/dev.143529.

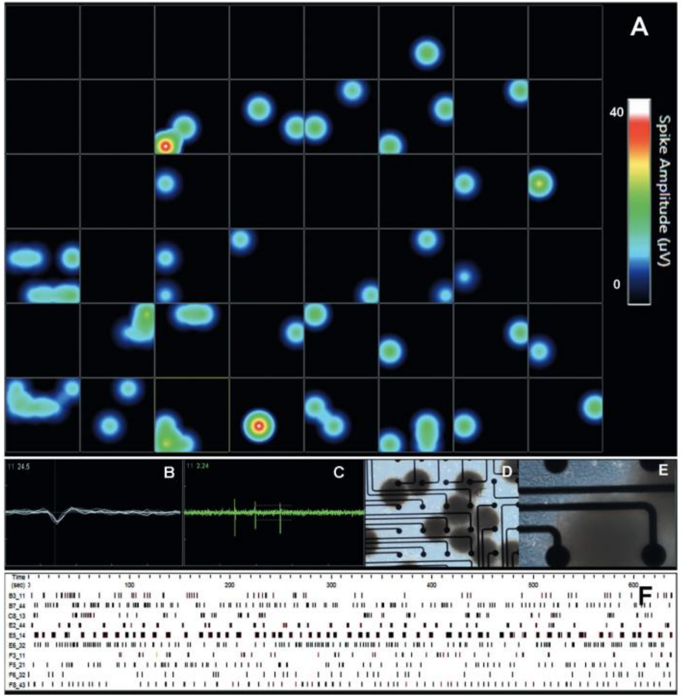
Embryoids, organoids and gastruloids: new approaches to understanding embryogenesis

Mijo Simunovic [1, 2], Ali H Brivanlou [3]

PMID: 28292844 PMCID: PMC5358114 DOI: 10.1242/dev.143529

Human Neural Organoid Models





> ALTEX. 2017;34(3):362-376. doi: 10.14573/altex.1609122. Epub 2016 Nov 24.

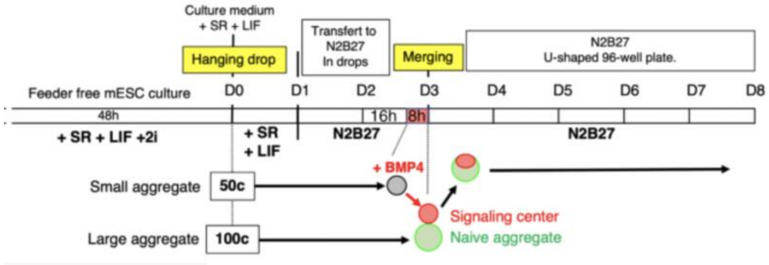
A human brain microphysiological system derived from induced pluripotent stem cells to study neurological diseases and toxicity

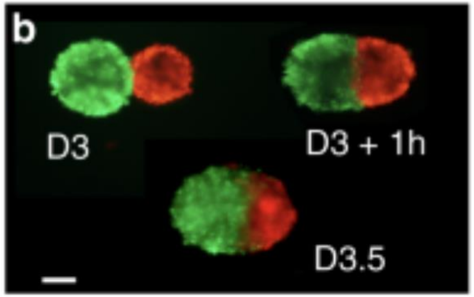
David Pamies [1], Paula Barreras [2,3], Katharina Block [1], Georgia Makri [2,4], Anupama Kumar [2,3], Daphne Wiersma [1], Lenna Smirnova [1], Ce Zang [2,4], Joseph Bressler [5], Kimberly M Christian [2,4], Georgina Harris [1], Guo-Li Ming [2,4,6], Cindy J Berlinicke [7], Kelly Kyro [8], Hongjun Song [2,4,6], Carlos A Pardo [2,3], Thomas Hartung [1,9], Helena T Hogberg [1]

PMID: 27883356 PMCID: PMC6047513 DOI: 10.14573/altex.1609122

Embryoid Model

Morphological models of tissues or processes
Embryoid body, bioprinted organization, geometrically constrained



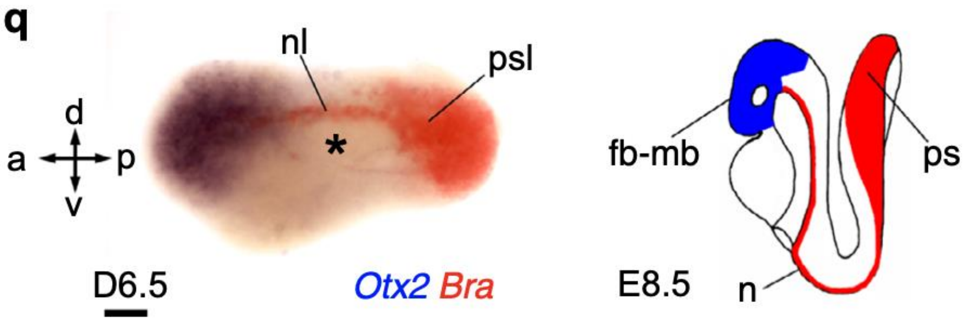


Article | Open Access | Published: 02 June 2021

Construction of a mammalian embryo model from stem cells organized by a morphogen signalling centre

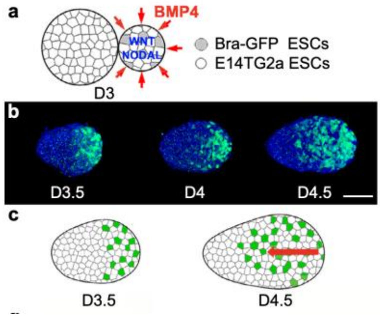
Peng-Fei Xu, Ricardo Moraes Borges, Jonathan Fillatre, Maraysa de Oliveira-Melo, Tao Cheng, Bernard Thisse & Christine Thisse

Nature Communications **12**, Article number: 3277 (2021) | Cite this article

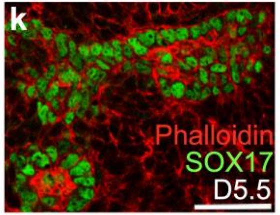


https://www.nature.com/articles/s41467-021-23653-4

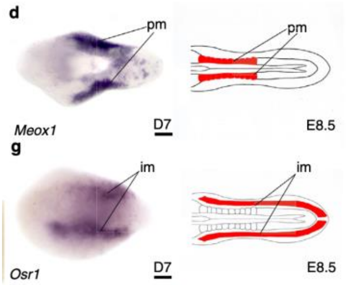
Embryoid Model



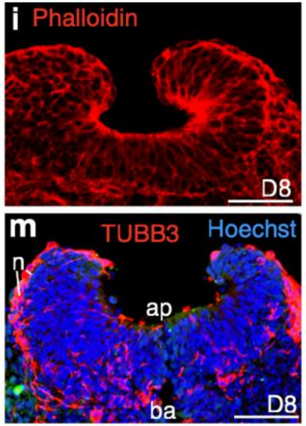
Endoderm



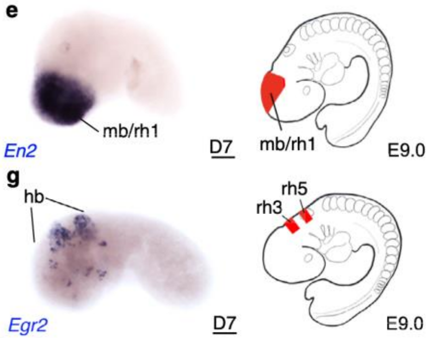
Mesoderm



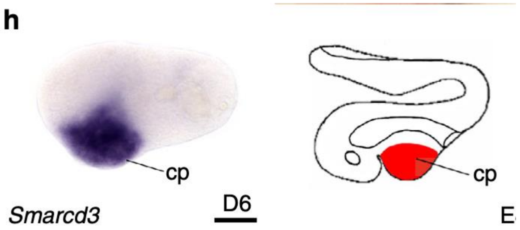
Neural Epithelium



Neuroectoderm



Cardiac



Article | Open Access | Published: 02 June 2021

Construction of a mammalian embryo model from stem cells organized by a morphogen signalling centre

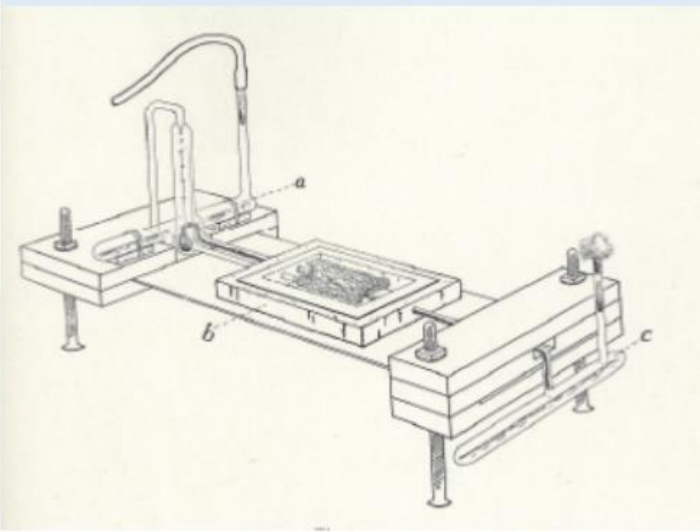
Peng-Fei Xu, Ricardo Moraes Borges, Jonathan Fillatre, Maraysa de Oliveira-Melo, Tao Cheng, Bernard Thisse & Christine Thisse

Nature Communications 12, Article number: 3277 (2021) | Cite this article

Tissue Chip-based Model

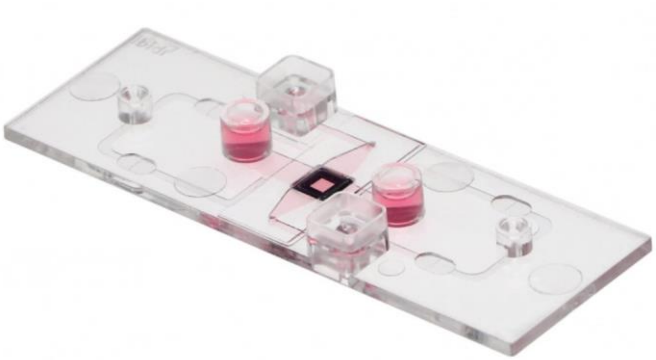
Constructed models that mimic tissue-level organization and physiology
Recapitulate a tissue
Establish progenitor model for morphogenesis

1912



Burrows, 1912

2022



https://ibidi.com

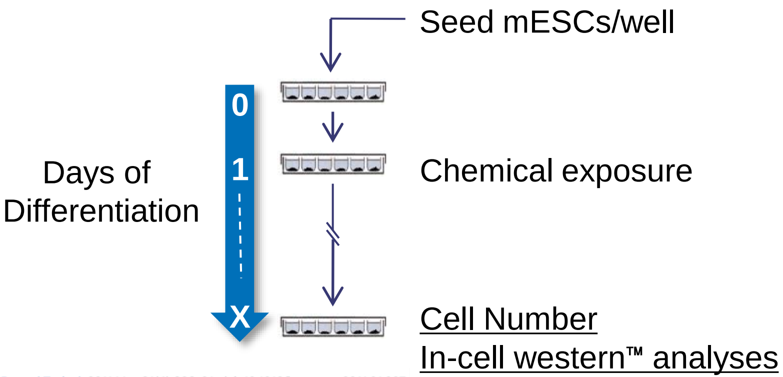
Application of Complex Models to Identify Putative Developmental Toxicants

70-90% of chemicals that affect stem cell endpoints are developmental toxicants in vivo

- HOWEVER -

50 – 60% of developmental toxicants do not affect the stem cell endpoints evaluated

Mouse Stem Cell ACDC Assay



> Reprod Toxicol. 2011 May;31(4):383-91. doi: 10.1016/j.reprotox.2011.01.007.

Mouse embryonic stem cell adherent cell differentiation and cytotoxicity (ACDC) assay

Marianne Barrier [1], Susan Jeffay, Harriette P Nichols, Kelly J Chandler, Maria R Hoopes, Kimberly Slentz-Kesler, E Sidney Hunter 3rd

PMID: 21296659 DOI: 10.1016/j.reprotox.2011.01.007

Why did so may chemicals not affect stem cells endpoints?

- Differentiation Hypothesis
- Developmental Process Hypothesis
- Pregnancy Triad System Hypothesis

Developmental toxicity can be produced by adverse effects on the embryo, mother, or placenta. A pregnancy systems model will be necessary to accurately identify all potential developmental toxicants.

There is increasing evidence of developmental consequences of placentation defects

doi:10.1038/nature26002

Placentation defects are highly prevalent in embryonic lethal mouse mutants

Vicente Perez-Garcia[1,2*], Elena Fineberg[1,2*], Robert Wilson[3], Alexander Murray[1,2], Cecilia Icoresi Mazzeo[4], Catherine Tudor[4], Arnold Sienerth[1,2], Jacqueline K. White[4], Elizabeth Tuck[4], Edward J. Ryder[4], Diane Gleeson[4], Emma Siragher[4], Hannah Wardle-Jones[4], Nicole Staudt[4], Neha Wali[4], John Collins[4], Stefan Geyer[5], Elisabeth M. Busch-Nentwich[4,6], Antonella Galli[4], James C. Smith[3], Elizabeth Robertson[7], David J. Adams[4], Wolfgang J. Weninger[5], Timothy Mohun[3] & Myriam Hemberger[1,2]

Abnormal placenta assoicated dysmorphologies	% difference	*p* value
Growth/size phenotype	31.28	1.93E-06
Nervous system development	22.03	1.26E-06
Brain morphology	29.17	2.49E-04
Heart morphology	31.22	5.18E-05
Blood vessel morphology	23.62	3.17E-03
Subcutaneous edema	22.25	8.64E-03

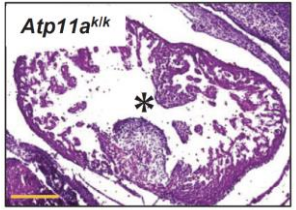
Modified from Perez-Garcia

PNAS RESEARCH ARTICLE | CELL BIOLOGY OPEN ACCESS

Inefficient development of syncytiotrophoblasts in the *Atp11a*-deficient mouse placenta

Yuki Ochiai[a], Chigure Suzuki[b,c], Katsumori Segawa[a,1], Yasuo Uchiyama[b,c], and Shigekazu Nagata[a,d,2]

Contributed by Shigekazu Nagata; received January 12, 2022; accepted March 22, 2022; reviewed by Raymond Birge and Yasushi Hirota

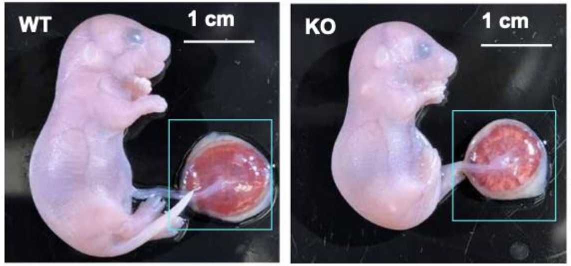


SCIENCE ADVANCES | RESEARCH ARTICLE

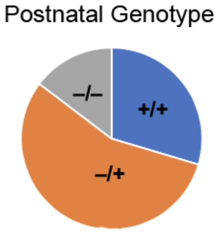
CELL BIOLOGY

TMEM16F phospholipid scramblase mediates trophoblast fusion and placental development

Yang Zhang[1,2], Trieu Le[1], Ryan Grabau[3], Zahra Mohseni[4], Hoejeong Kim[5], David R. Natale[6], Liping Feng[4,7], Hua Pan[3], Huanghe Yang[1,2*]

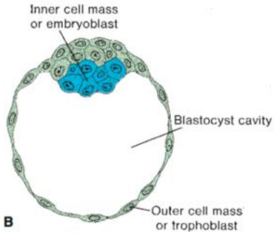


Modified from Zhang

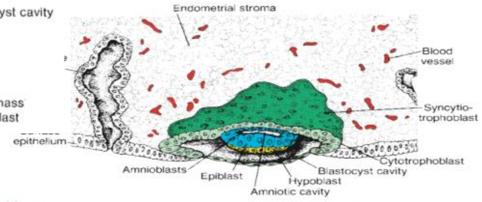


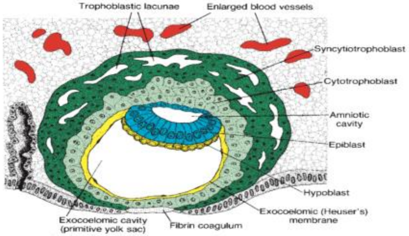
Modified from Zhang

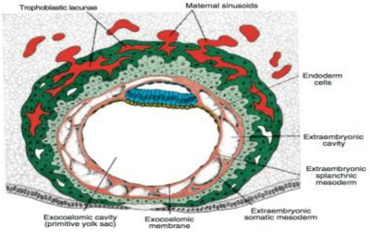
At the time of Implantation, the conceptus consists of the inner cell mass and the surrounding trophoblast layer

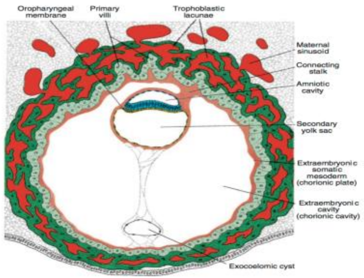


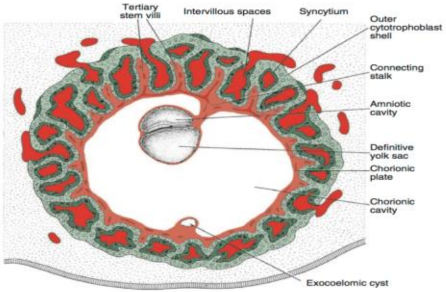
Placental Development

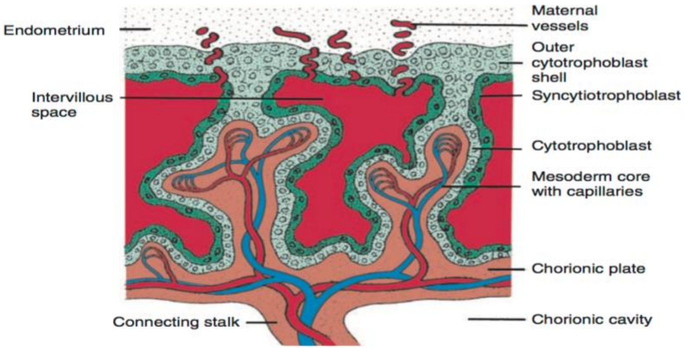






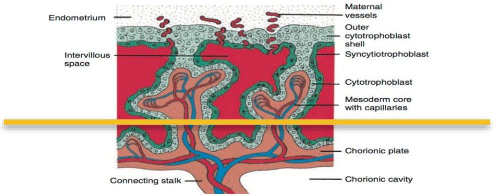


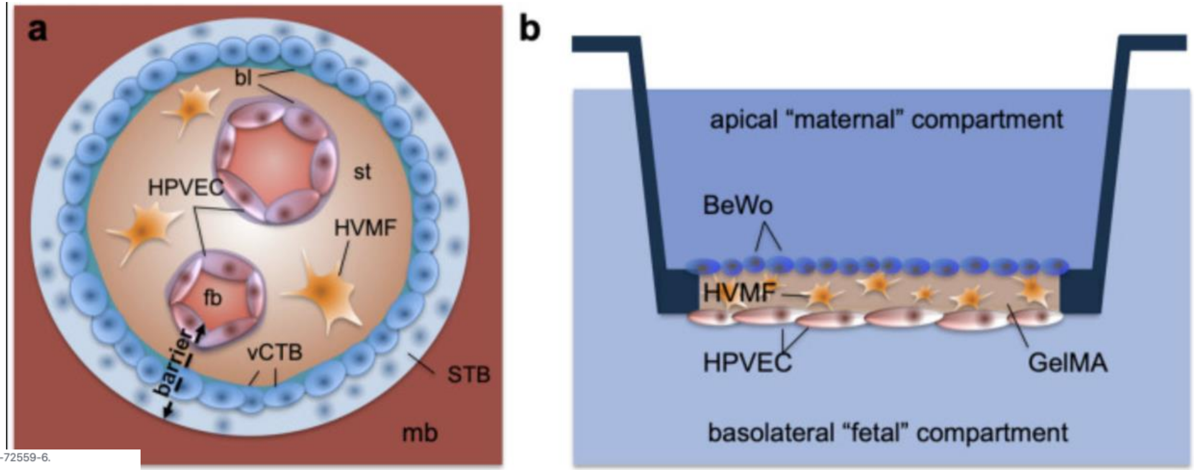




Sadler. Langman's Medical Embryology. 12th Edition

Placental Models for Developmental Toxicity studies





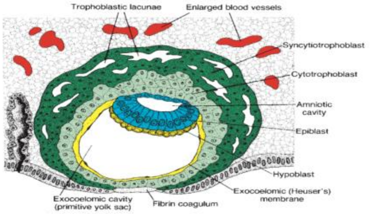
> Sci Rep. 2020 Sep 24;10(1):15606. doi: 10.1038/s41598-020-72559-6.

Inspired by the human placenta: a novel 3D bioprinted membrane system to create barrier models

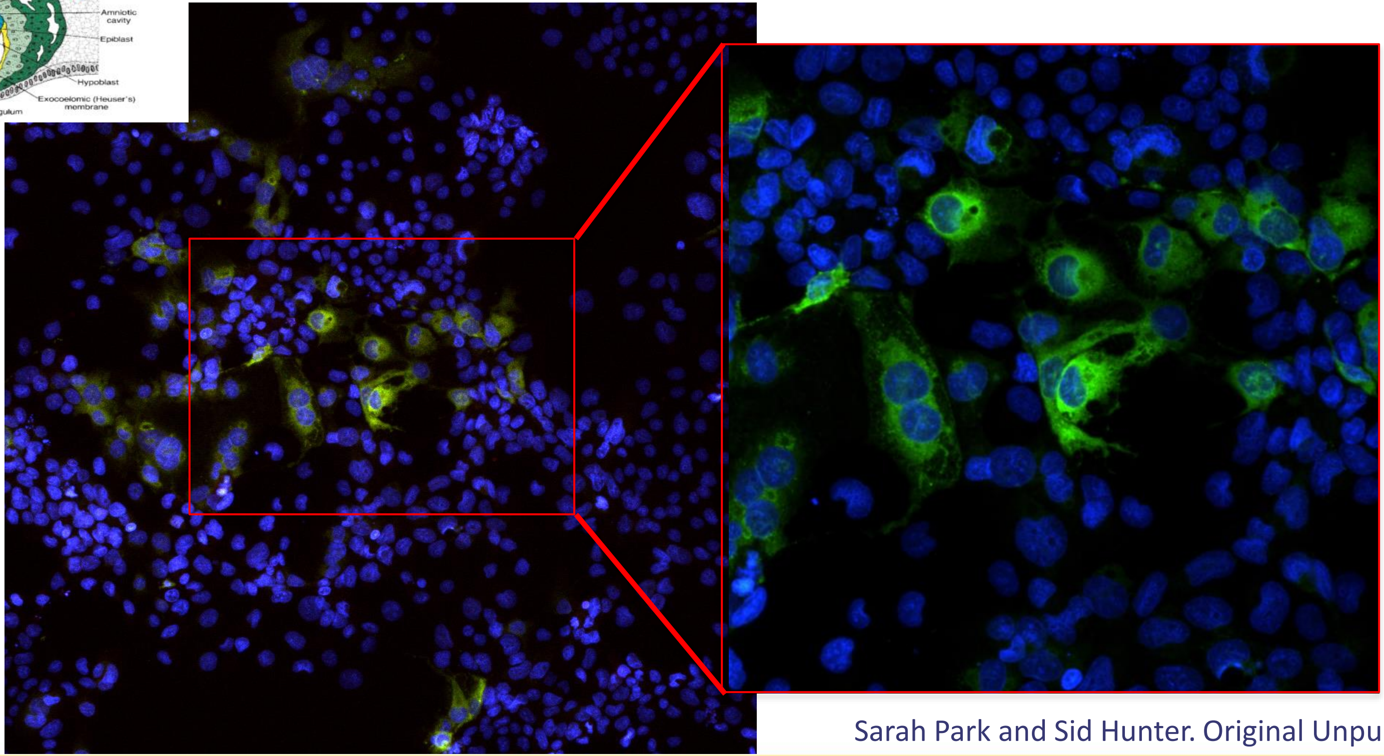
Anna-Elisabeth Kreuder [1, 2], Aramis Bolaños-Rosales [3, 4], Christopher Palmer [4], Alexander Thomas [3, 4], Michel-Andreas Geiger [4], Tobias Lam [4], Anna-Klara Amler [3, 4], Udo R Markert [5], Roland Lauster [3], Lutz Kloke [6]

https://www.ted.com/talks/anna_elisabeth_kreuder_bioprinting_the_human_placenta

Placental Models for Developmental Toxicity studies



BeWo Choriocarcinoma Cells syncitialization as a model of early placental development



Blue – Nuclei
Green - hCG

Sarah Park and Sid Hunter. Original Unpublished figure

Chemicals affect Cytotrophoblasts and mouse Stem Cells

Chemical	Cytotrophoblast Cell Death (LDH)	Cytotrophoblast Live Cell (MTS)	Cytotrophoblast (hCG)	mESC ACDC Assessment GSC Differentiation	mESC ACDC Assessment Cytotoxicity
5-Fluorouracil	10	10	10	0.7	0.5
6-aminonicotinamide	1	1	1	8	8
Tributyltin	0.5	0.5	0.5	0.02	0.2
Diphenhydramine HCl	100	-	-	-	--
Diphenylhydantoin	-	-	-	-	-
Sulfasalazine	-	-	-	-	-
Carbamazepine	-	-	-	-	-
Busulfan	-	-	-	20	-
Tebuconazole	100	-	30	-	-
Butylparaben	100	100	100	-	-
Zearalenone	100	100	30	No Data	No Data
TBBPA	-	-	100	No Data	No Data
Braga (TMEM16F)					
Triclosan	30	100	30	20.4	20.4
Simvastatin	30	30	2	-	-
Promethazine	30	30	30	No Data	No Data
Paroxetine	10	30	30	No Data	No Data

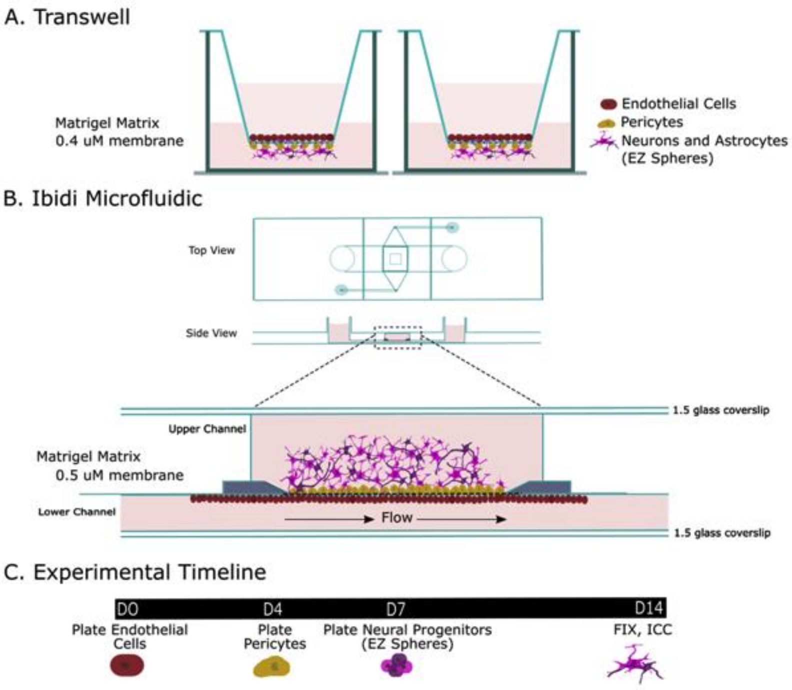
- No Effect, Chemical Concentrations (µM)

Sarah Park and Sid Hunter. Original Unpublished Data

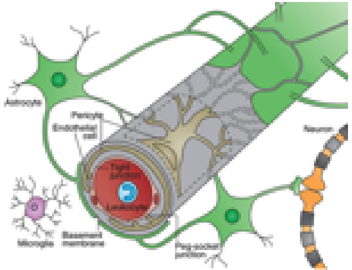
Organotypic Culture model of Early Human Brain development

3D Static

3D Dynamic



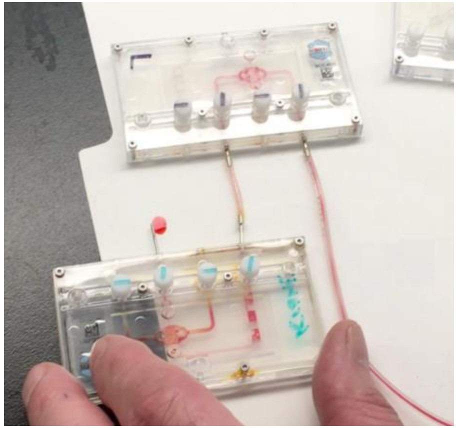
NeuroVascular Unit



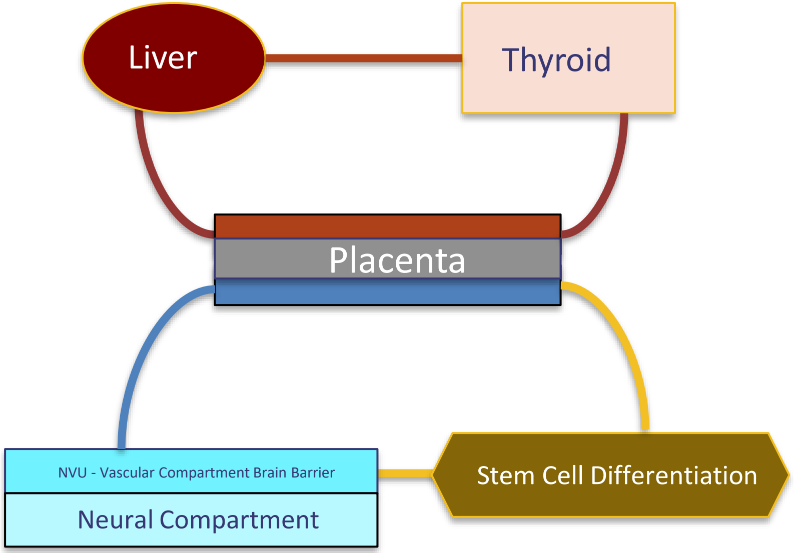
Obermeyer et al., 2013

Andrew Schwab et al., Submitted for publication

Next Generation: Integrated Systems Model



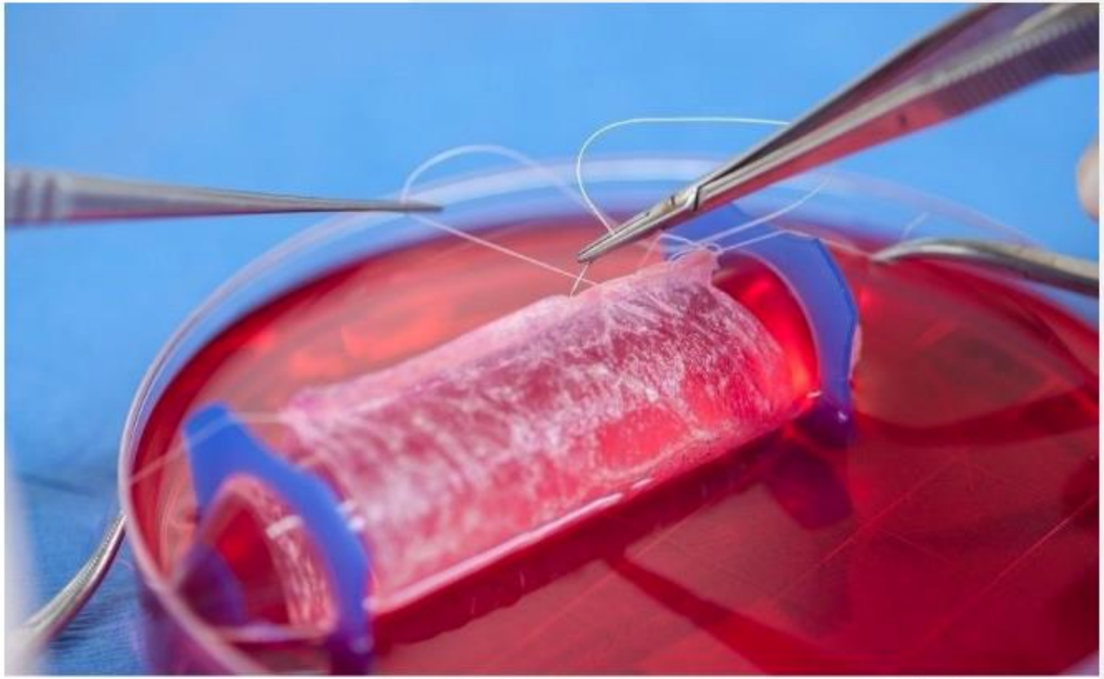
Edward Kelly and David Eaton
University of Washington



Multi-chip dual flow system design

V. Richardson - Original Image

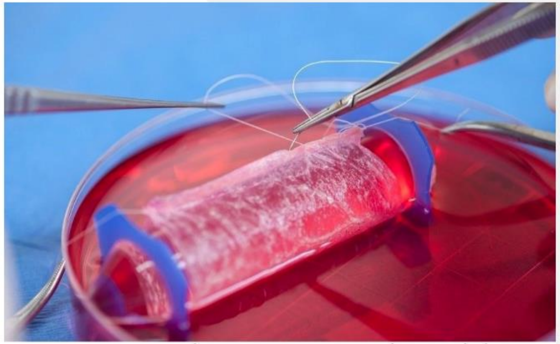
Tissue-Engineered Uterus Supports Live Birth in Rabbits



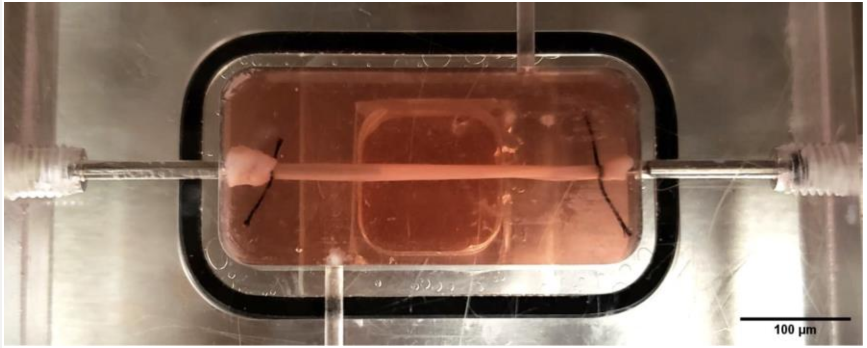
https://health-innovations.org/2020/07/03/a-tissue-engineered-uterus-supports-live-births-in-rabbits/

Magalhaes, R.S., Williams, J.K., Yoo, K.W. *et al.* A tissue-engineered uterus supports live births in rabbits. *Nat Biotechnol* (2020). https://doi.org/10.1038/s41587-020-0547-7

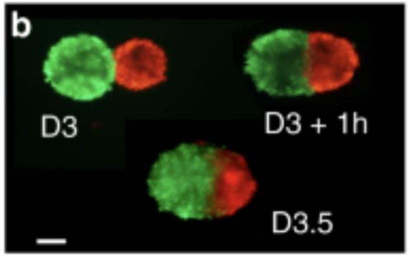
Is it possible that combining the tools used to create blood vessels with tissue engineering approach for a uterus could establish an in vitro model for pregnancy model and embryonic development



https://health-innovations.org/2020/07/03/a-tissue-engineered-uterus-supports-live-births-in-rabbits/



https://bme.duke.edu/about/news/engineered-living-cell-blood-vessel-provides-new-insights-progeria

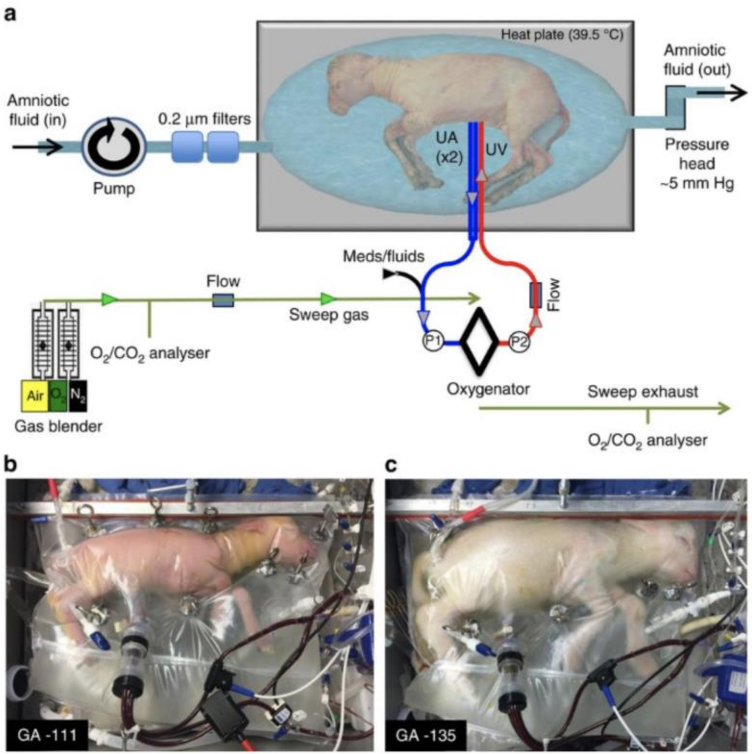


https://www.nature.com/articles/s41467-021-23653-4

Hunter. Original

Aldous Huxley's Brave New World???

Figure 1: UA/UV Biobag system design.



Open Access | Published: 25 April 2017

An extra-uterine system to physiologically support the extreme premature lamb

Emily A. Partridge, Marcus G. Davey, Matthew A. Hornick, Patrick E. McGovern, Ali Y. Mejaddam, Jesse D. Vrecenak, Carmen Mesas-Burgos, Aliza Olive, Robert C. Caskey, Theodore R. Weiland, Jiancheng Han, Alexander J. Schupper, James T. Connelly, Kevin C. Dysart, Jack Rychik, Holly L. Hedrick, William H. Peranteau & Alan W. Flake

Nature Communications **8**, Article number: 15112 (2017) | Cite this article


What opportunities will come today!
Hunter. Original

References

T. W. Sadler. Langman’s Medical Embryology, 8th Ed., Lippincott Williams and Wilkin, Baltimore, MD, USA, 2000

Hutson, M.S., Leung, M.C.K., Baker, N.C., Spencer, R.M., Knudsen, T.B. (2017). Chem Res. Toxicol. 30: 965-979. DOI: /10.1021/acs.chemrestox.6b00350

Pamies, D., Barreras, P., Block, K., Makri, G., Kumar, A., Wiersma, D., Smirnova, L., Zhang, C., Bressler, J., Christian, K. M., Harris, G., Ming, G.- li, Berlinicke, C. J., Kyro, K., Song, H., Pardo, C. A., Hartung, T. and Hogberg, H. T. (2017) *ALTEX - Alternatives to animal experimentation*, 34(3), 362–376. doi: 10.14573/altex.1609122.

Karzbrun, E., Khankhel, A.H., Megale, H.C. Glasauer, S.M.K. Wyle, Y. Britton, G., Warmflash, A. Kosik, K.S., Siggia, E.D., Shraiman, B.I., Streichan, S.J. (2021). Nature 599(7884):268-272. doi: 10.1038/s41586-021-04026-9.

Schwab, A.J., Jeffay, S.C., Nichols, H.P., Hunter, 3rd, E.S. (2022) Submitted for publication

Simunovic M, Brivanlou AH. Embryoids, organoids and gastruloids: new approaches to understanding embryogenesis. Development. 2017 Mar 15;144(6):976-985. doi: 10.1242/dev.143529. PMID: 28292844; PMCID: PMC5358114.

Obermeier, B., Daneman, R. & Ransohoff, R. Development, maintenance and disruption of the blood-brain barrier. *Nat Med* **19,** 1584–1596 (2013). https://doi.org/10.1038/nm.3407

Magalhaes, R.S., Williams, J.K., Yoo, K.W. *et al.* A tissue-engineered uterus supports live births in rabbits. *Nat Biotechnol* (2020). https://doi.org/10.1038/s41587-020-0547-7

Bedzhov, I., Zernicka-Goetz, M. (2014). Self-Organizing Properties of Mouse Pluripotent Cells Initiate Morphogenesis upon Implantation. *Cell*. 156: 1032–1044

Deglinverti, A, Croft, GF., Pietila, LN, Zernicka-Goetz, M., Siggia, ED, Brivanlou, AH. (2016). Self-Organization of the *In Vitro* Attached Human Embryo. *Nature*. 533: 251–254.

References

Reviews:

Sonia Youhanna, Aurino M. Kemas, Lena Preiss, Yitian Zhou, Joanne X. Shen, Selgin D. Cakal, Francesco S. Paqualini, Sravan K. Goparaju, Reza Zandi Shafagh, Johan Ulrik Lind, Carl M. Sellgren and Volker M. Lauschke. Organotypic and Microphysiological Human Tissue Models for Drug Discovery and Development—Current State-of-the-Art and Future Perspectives Pharmacological Reviews January 1, 2022, 74 (1) 141-206; DOI: https://doi.org/10.1124/pharmrev.120.000238

Sura R, Van Vleet T, Berridge BR. (2020). Microphysiological Systems: A Pathologist's Perspective. *Veterinary Pathology*. 2020;57(3):358-368. doi:10.1177/0300985820908794

Minghui Li, Jing Gong, Lixiong Gao, Ting Zou, Jiahui Kang, Haiwei Xu, (2022). Advanced human developmental toxicity and teratogenicity assessment using human organoid models. Ecotoxicology and Environmental Safety, 235, 113429, https://doi.org/10.1016/j.ecoenv.2022.113429.

Truskey George A. (2018). Human Microphysiological Systems and Organoids as in Vitro Models for Toxicological Studies. Frontiers in Public Health, DOI=10.3389/fpubh.2018.00185

Luz AL, Tokar EJ. Pluripotent Stem Cells in Developmental Toxicity Testing: A Review of Methodological Advances. Toxicol Sci. 2018 Sep 1;165(1):31-39. doi: 10.1093/toxsci/kfy174. PMID: 30169765; PMCID: PMC6111785.