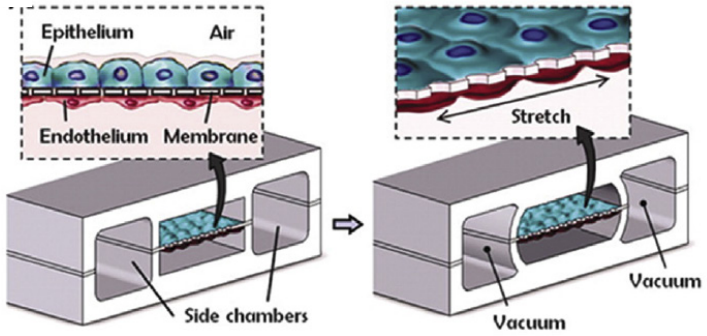
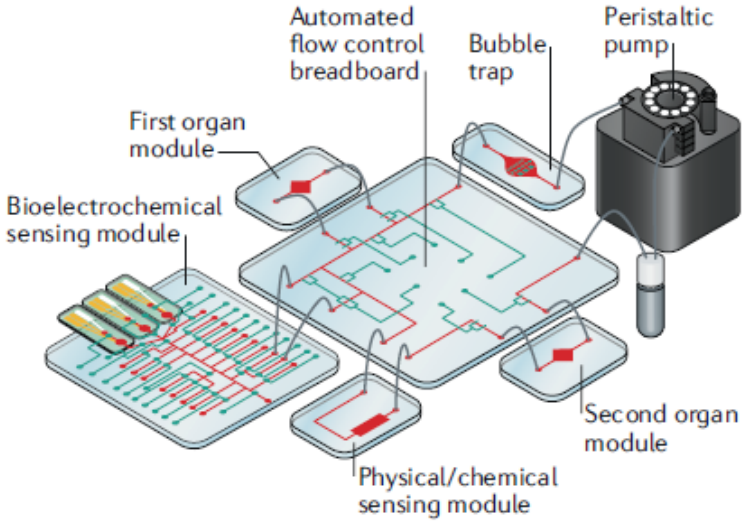
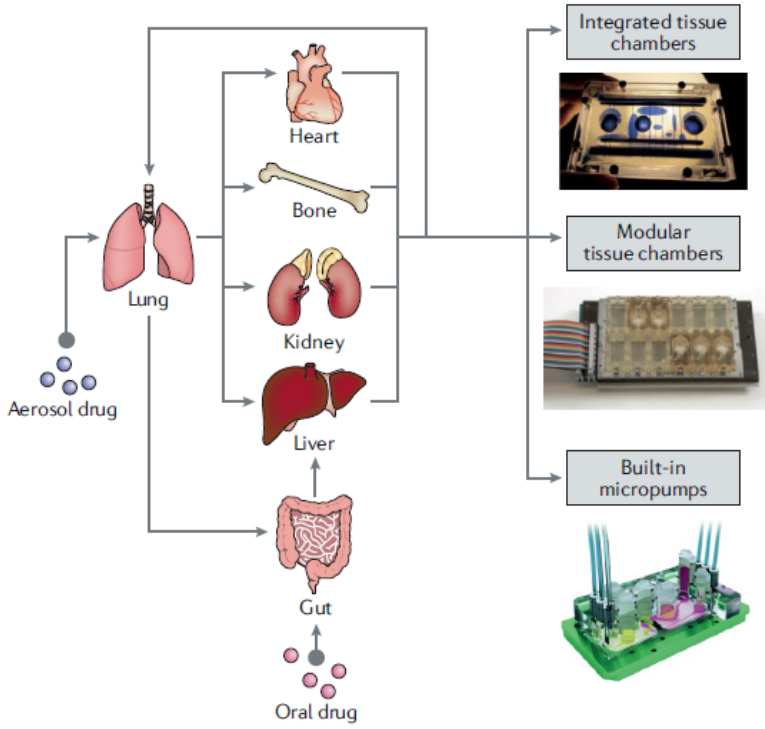
Introduction to BioMEMS & Medical Microdevices

Organ-on-a-Chip (OOCs)

Prof. Steven S. Saliterman, http://saliterman.umn.edu/





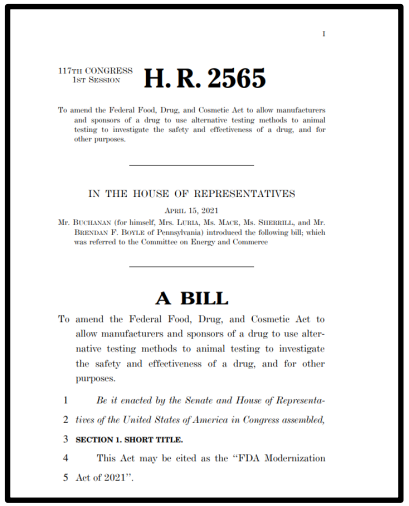


FDA Modernization Act of 2021

- A bill to amend the *Federal Food, Drug, and Cosmetic Act* to allow manufacturers and sponsors of a drug to use “alternative testing methods to animal testing” to investigate the “safety and effectiveness of a drug,” and for other purposes.
- Broadening the scope of “acceptable preclinical models” for drug development.
- Enabling researchers to test a drug’s safety and efficacy using more advanced and humane methods in “place of animals” whenever possible.

Applause applause!

- The Act strikes “animal” and inserts “nonclinical tests or studies.”
- The bill defines “nonclinical tests or studies” as a test or study that is most likely to predict human response based on scientific evidence and occurs before or during the clinical trial phase of the investigation of the safety and effectiveness of a drug.
- Such test or study may include the following:
  - Cell-based assays
  - Organ chips and microphysiological systems
  - Sophisticated computer modeling
  - Other human biology-based test methods



117TH CONGRESS
1ST SESSION

H. R. 2565

To amend the Federal Food, Drug, and Cosmetic Act to allow manufacturers and sponsors of a drug to use alternative testing methods to animal testing to investigate the safety and effectiveness of a drug, and for other purposes.

IN THE HOUSE OF REPRESENTATIVES

APRIL 15, 2021

Mr. BUCHANAN (for himself, Mrs. LUNA, Ms. MACE, Ms. SHERRILL, and Mr. BRENDAN F. BOYLE of Pennsylvania) introduced the following bill; which was referred to the Committee on Energy and Commerce

A BILL

To amend the Federal Food, Drug, and Cosmetic Act to allow manufacturers and sponsors of a drug to use alternative testing methods to animal testing to investigate the safety and effectiveness of a drug, and for other purposes.

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,

SECTION 1. SHORT TITLE.

This Act may be cited as the “FDA Modernization Act of 2021”.

Cost of Drug Development

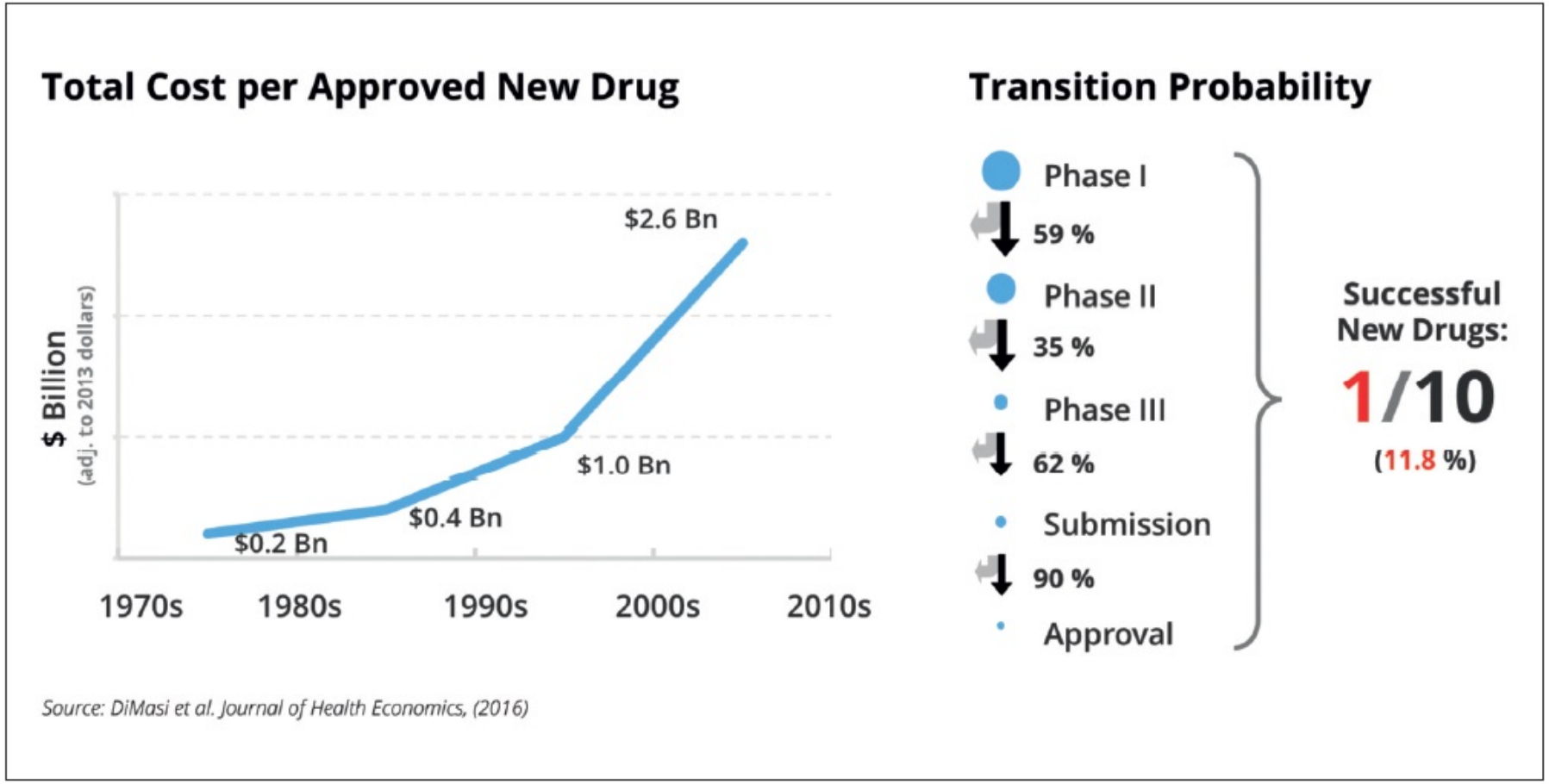


Figure 1 Costs of drug development have risen while overall probability of regulatory approval has reduced[1]. *Image taken from DiMasi JA et al. J Health Econ. 2016;47:20-33*

Organ-on-a-Chip Research

Steven S. Saliterman

https://youtu.be/5I0QVeFbS0c

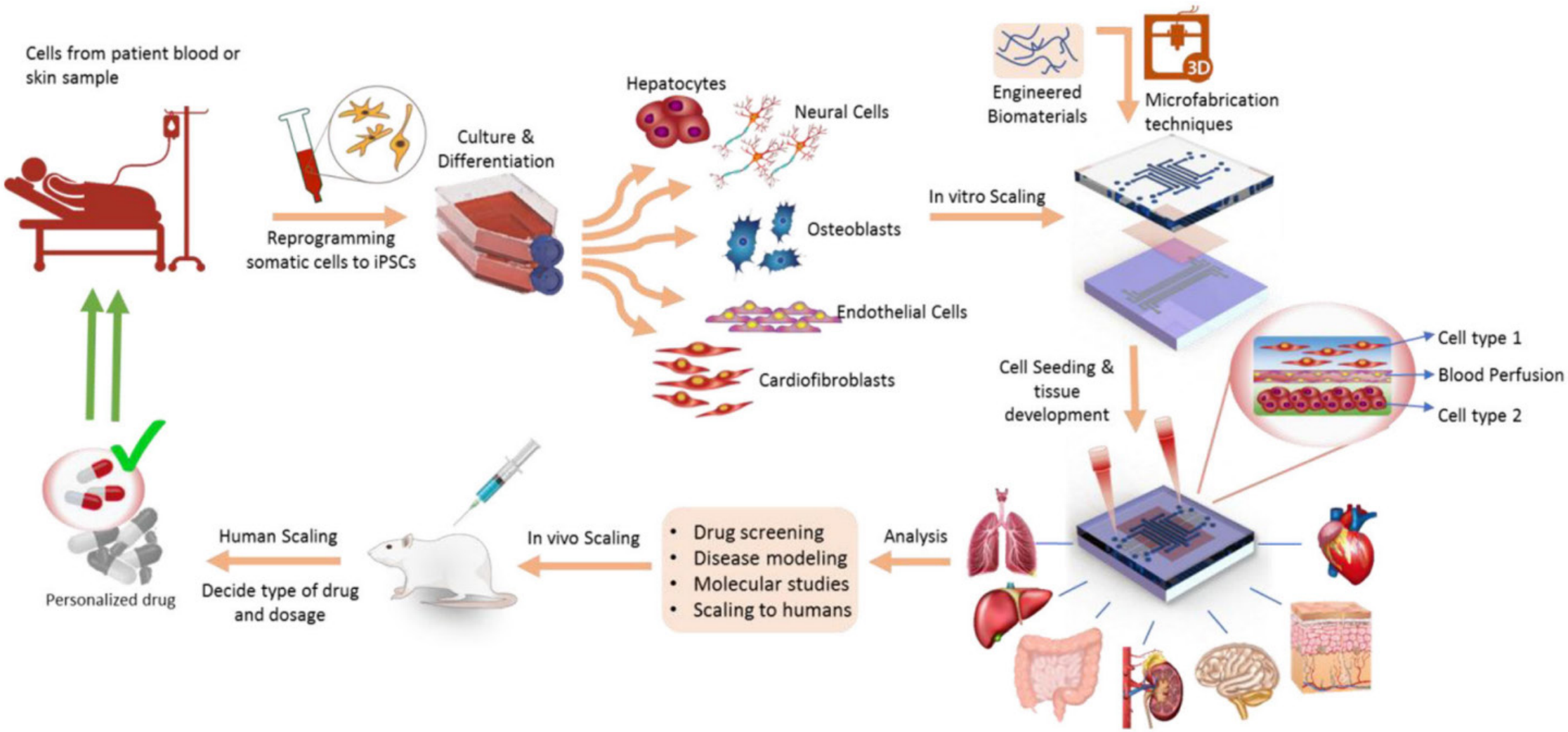
Why Organ-on-a-Chip Technology?

- Drug discovery and cancer modeling – need for advanced, preclinical models.
  - Traditionally animals have been used.
    - Ethical and species differences concerns.
    - Errant pharmacokinetic predictions.
    - Regulations may prohibit testing – ie. cosmetics testing in EU since 2013.
  - Cell-based assays using human derived cells have been an alternative approach.
    - Cells often do not retain their original organ function and morphology.
    - Difficult to predict drug efficacy, toxicity and organ interactions.

Kimura H, Sakai Y, Fujii T. Organ/body-on-a-chip based on microfluidic technology for drug discovery. *Drug Metabolism and Pharmacokinetics.* 2018;33(1):43-48.
Zhang B, Korolj A, Lai BFL, Radisic M. Advances in organ-on-a-chip engineering. *Nature Reviews Materials.* 2018;3(8):257-278.

- Better replication of the pathophysiology of human disease.
- Better understanding of drug sensitivities for specific subsets of patients.
- ECM engineering for mimicking healthy and disease states.
- Assessment of environmental chemical teratogenic effects.

Cell Cycle for Personalized Medicine…



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Jodat YA, Kang MG, Kiaee K, et al. Human-Derived Organ-on-a-Chip for Personalized Drug Development. *Current pharmaceutical design*. 2018;24(45):5471-5486. doi:10.2174/1381612825666190308150055

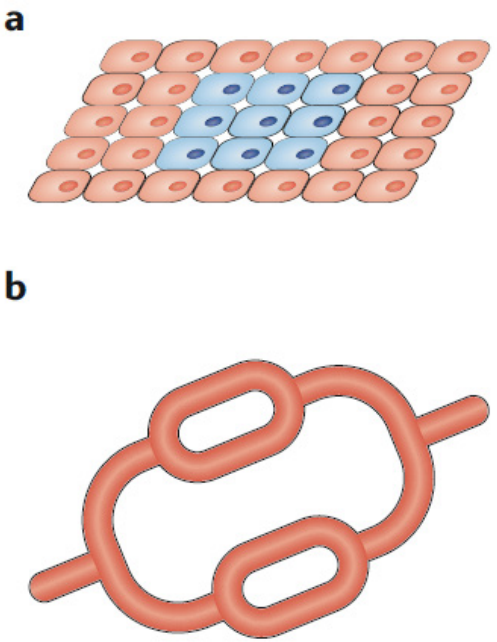
Requirements…

- Reproduction of specific tissue microenvironment and architecture.
  - The predictive value of a model is improved the more the species-specific biology is mimicked.
- Recreation of these aspects of human physiology:
  - Multicellular vascular or epithelial interfaces of organs (ie. blood vessels, lung & gut).
  - Tissue-level organization of parenchymal cells (liver, heart, muscles tumors etc.)
  - Interaction of multiple organs (ie. drug absorption, distribution, metabolism, and elimination).

Zhang B, Korolj A, Lai BFL, Radisic M. Advances in organ-on-a-chip engineering. *Nature Reviews Materials.* 2018;3(8):257-278.

Four Components of an Organ-on-a-Chip…

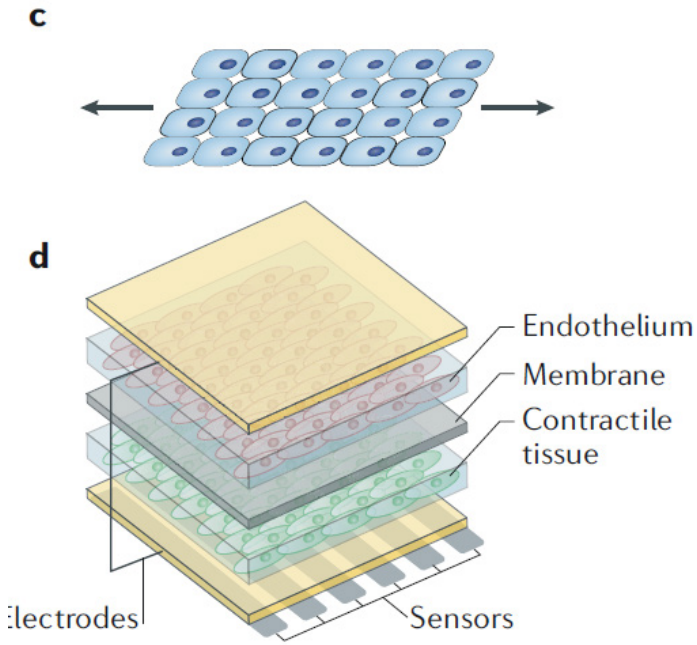
1. Geometric confinement and patterning:
   - Spatially defined multicellular co-culture.
   - Phenotypical change may be induced by physical confinement.
2. Control of flow:
   - Fluid inlets and outlets.
   - Physiological cell to liquid ratio.



E

Maoz, B. M. et al. Organs- on-Chips with combined multi- electrode array and transepithelial electrical resistance measurement capabilities. Lab. Chip 17, 2294–2302 (2017).

3. Environmental control (c).
   - Mechanical stimulation and actuation.
   - Electrical stimulation.
   - Environmental control of O_2, CO_2, pH, nutrients and growth factors.
   - On demand presence of drugs or toxins.
4. Sensors and physiologic readouts (d).
   - Built-in electrodes.
   - Optical readouts.
   - Online sensors for biochemical readouts.



Maoz, B. M. et al. Organs- on-Chips with combined multi- electrode array and transepithelial electrical resistance measurement capabilities. Lab. Chip 17, 2294–2302 (2017).

Methods

- 3D printing and microfabrication.
- Incorporating multiple cell types.
- Guided spacial confinement of cells.
- Use of sensors and microfluidic channels.
- Contrast this to an *organoid approach* which relies on the spontaneous self-assembly of cells to achieve complex tissue and organ-level organization and function.

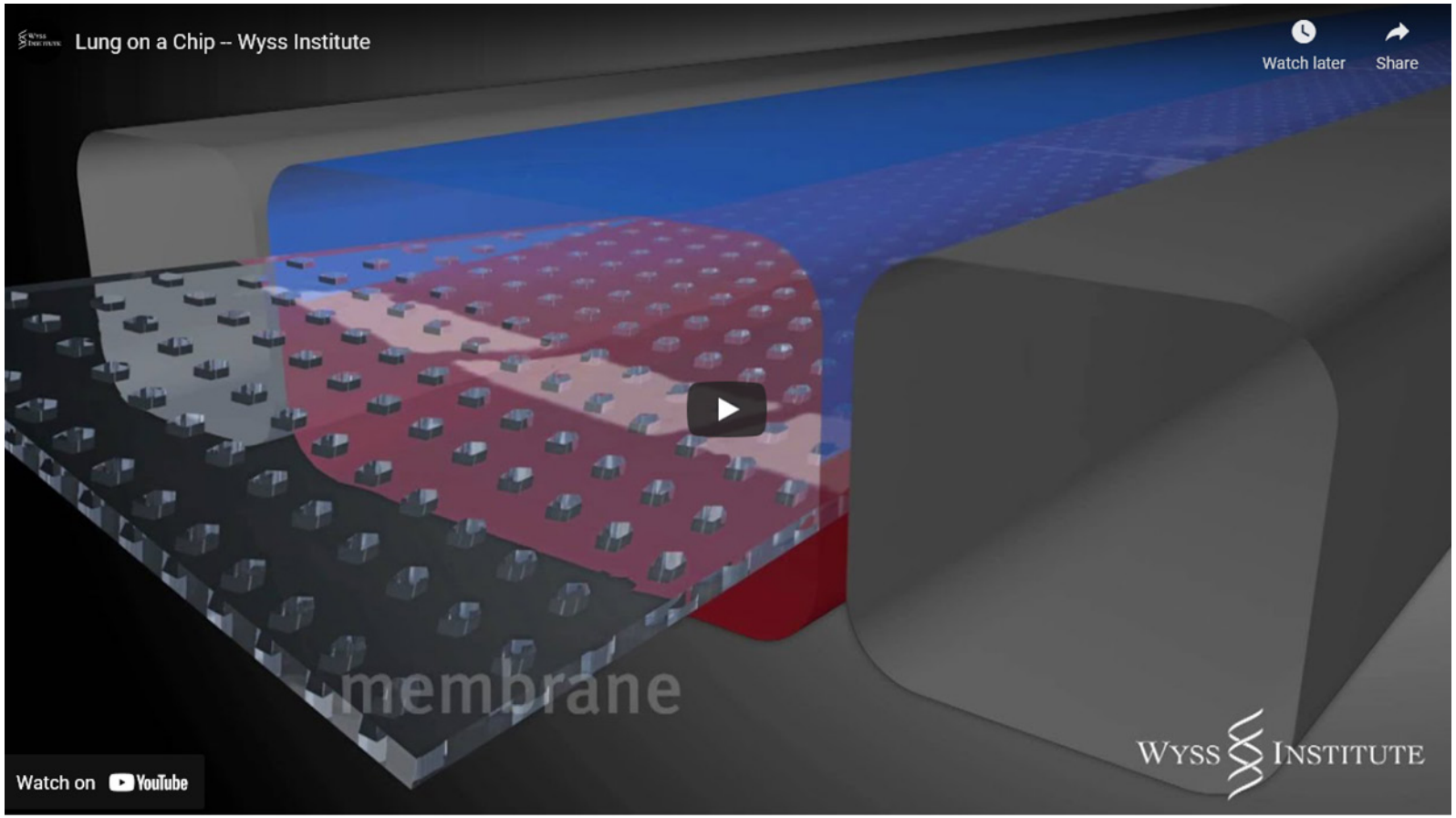
Zhang B, Korolj A, Lai BFL, Radisic M. Advances in organ-on-a-chip engineering. *Nature Reviews Materials.* 2018;3(8):257-278.

What's Required for Organ-Specific Structures?

- Epithelial Barriers
  - For lung, airway, gut, kidney proximal tubules and glomeruli, and placenta.
- Vascular and Lymphatic Barriers
  - For blood-brain and blood-retina barriers, microvasculature, artery, and lymphatic vessels
- Parenchymal Tissues
  - For myocardium, skeletal muscles, liver, tumors, adipose tissue and peripheral nerves.

Zhang B, Korolj A, Lai BFL, Radisic M. Advances in organ-on-a-chip engineering. *Nature Reviews Materials.* 2018;3(8):257-278.

Lung-on-a-Chip…



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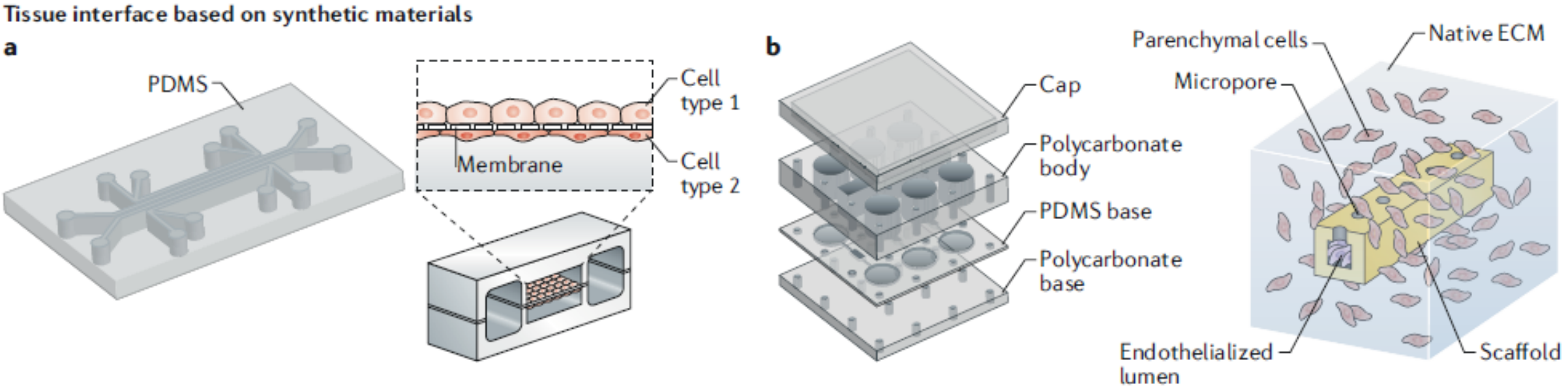
Reproducing the Tissue Barrier Function…



PDMS membranes. (e.g., Lung)

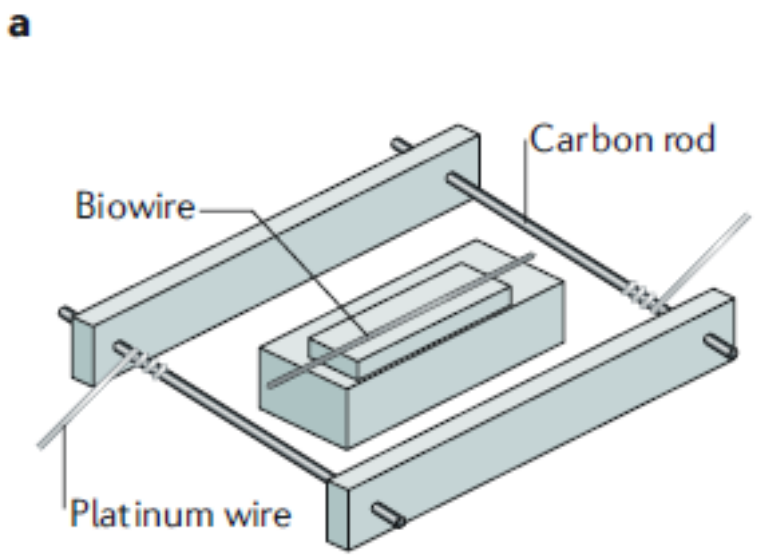
Perfusion bioreactor and synthetic microfabricated scaffold. (Scaffold with Vasculature)

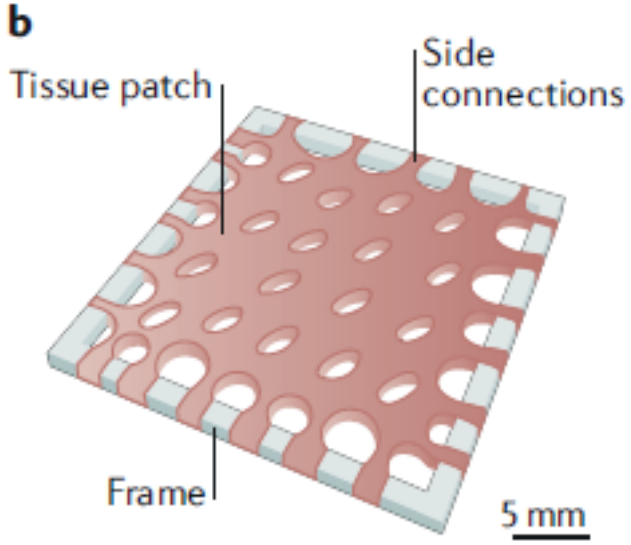
See Huh & Zang articles:

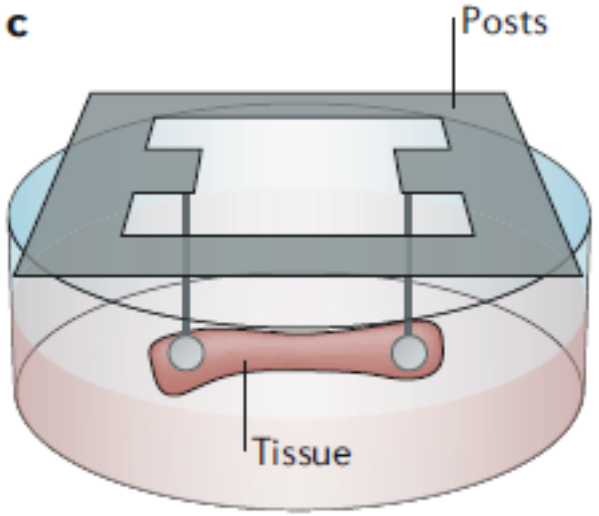
a) Huh, D. et al. Reconstituting organ- level lung functions n a chip. Science 328, 1662–1668 (2010).
b) Zhang, B. et al. Biodegradable scaffold with built- in vasculature for organ- on-a- chip engineering and direct surgical anastomosis. Nat. Mater. 15, 669–678 (2016).

Reproducing Elongated Parenchymal Tissues…

Cardiomyocytes…





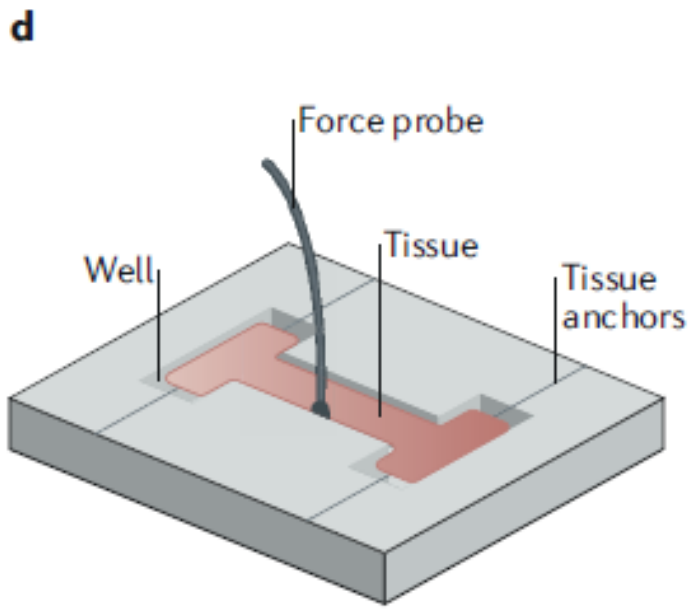


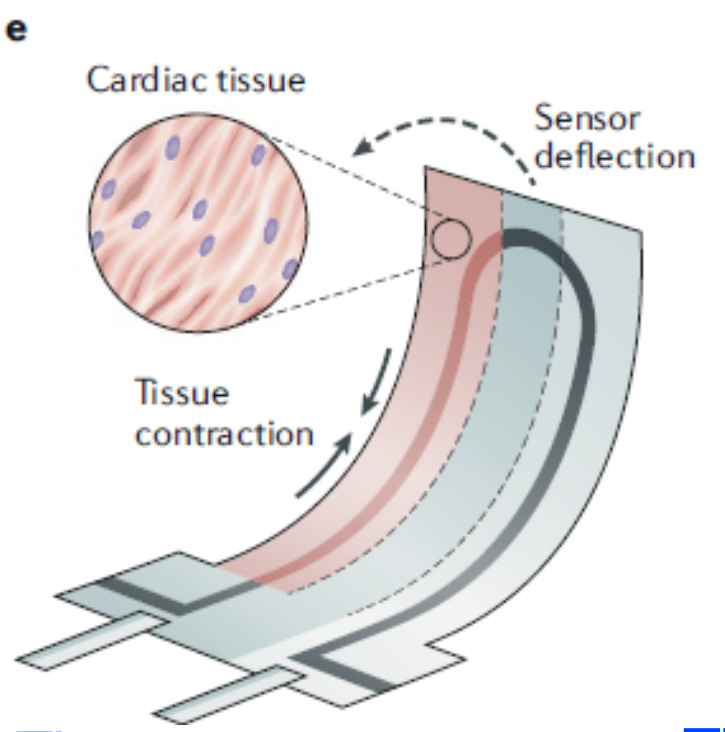
Tissue compact along wires.

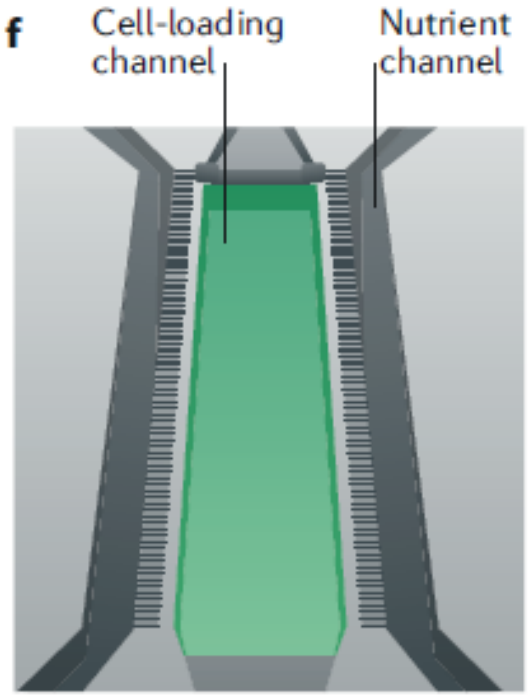
Posts can anchor a tissue network.

Tissues can hang upside down on pillars.

a) Sun, X. & Nunes, S. S. Biowire platform for maturation of human pluripotent stem cell- derived cardiomyocytes Methods 101, 21–26 (2016).
b) Bian, W., Badie, N., Himel IV, H. D. & Bursac, N Robust T- tubulation and maturation of cardiomyocytes using tissue- engineered epicardial mimetics. Biomaterials 35, 3819–3828 (2014).
c) Stoehr, A. et al. Spontaneous formation of extensive vessel- like structures in murine engineered heart tissue. Tissue Eng. Part A 22, 326–335 (2016).







Tissues bridging across parallel rods.

Tissue grown on a flexible cantilever.

Tissue grown along patterned channels and grooves.

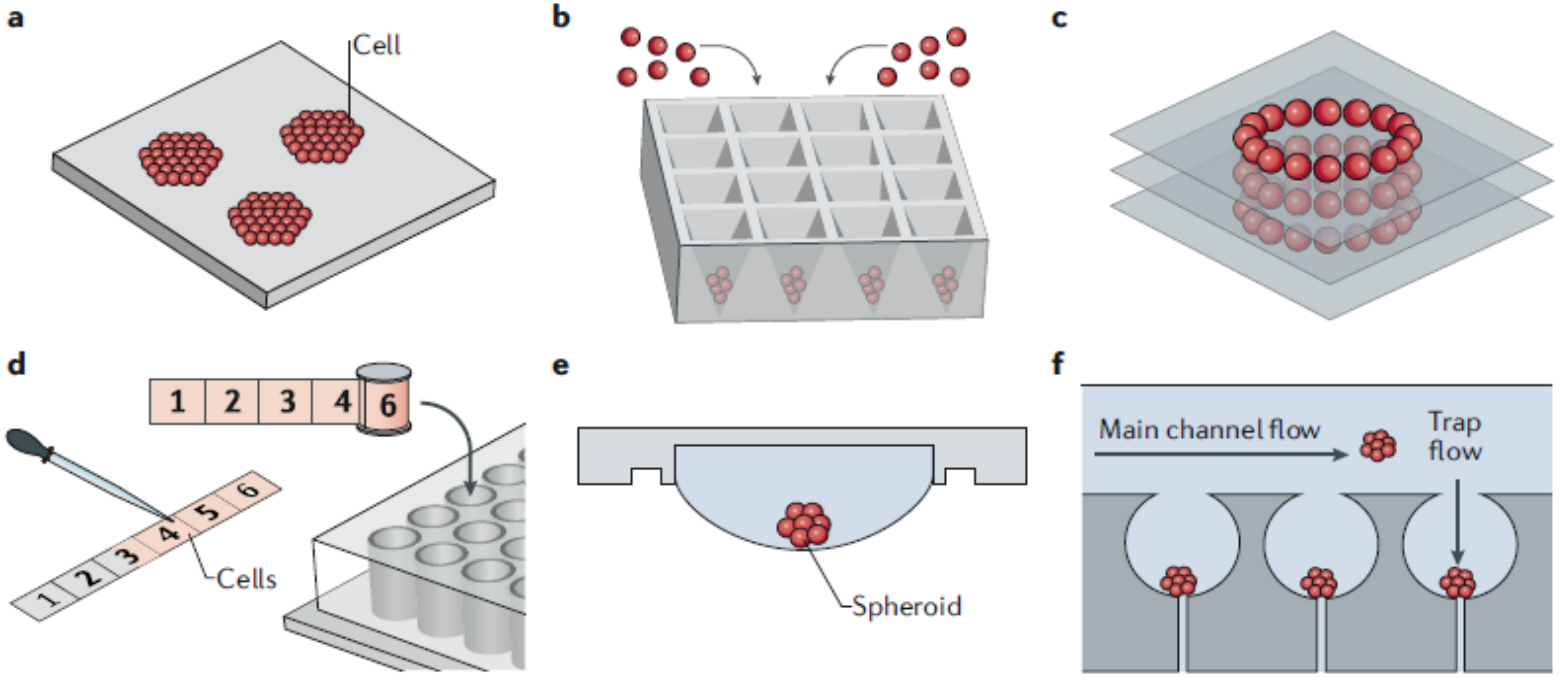
d) Schroer, A. K., Shotwell, M. S., Sidorov, V. Y. Wikswo, J. P. & Merryman, W. D. I- Wire Heart- on-a- Chip II: biomechanical analysis of contractile, three- dimensional cardiomyocyte tissue constructs. Acta Biomaterialia 48, 79–87 (2017).
e) Lind, J. U. et al. Instrumented cardiac microphysiological devices via multimaterial three- dimensional printing. Nat. Mater. 16, 303–308 (2016).
f) Mathur, A. et al. Human iPSC- based cardiac microphysiological system for drug screening applications. Sci. Rep. 5, 8883 (2015).

Reproducing Spherical Parenchymal Tissues…

Micro-patterned Clusters

Inverted Pyramidal Wells

3D Multilayer Printing



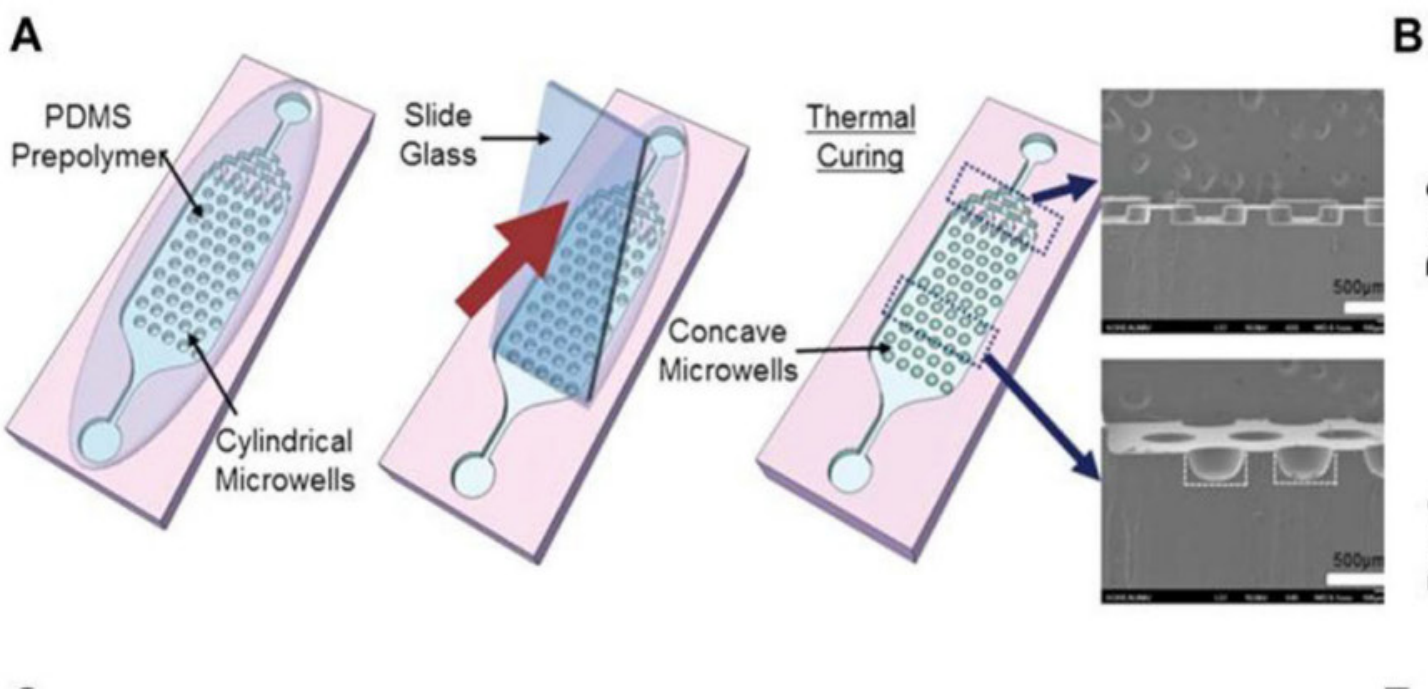
Rolled-up Scaffolds

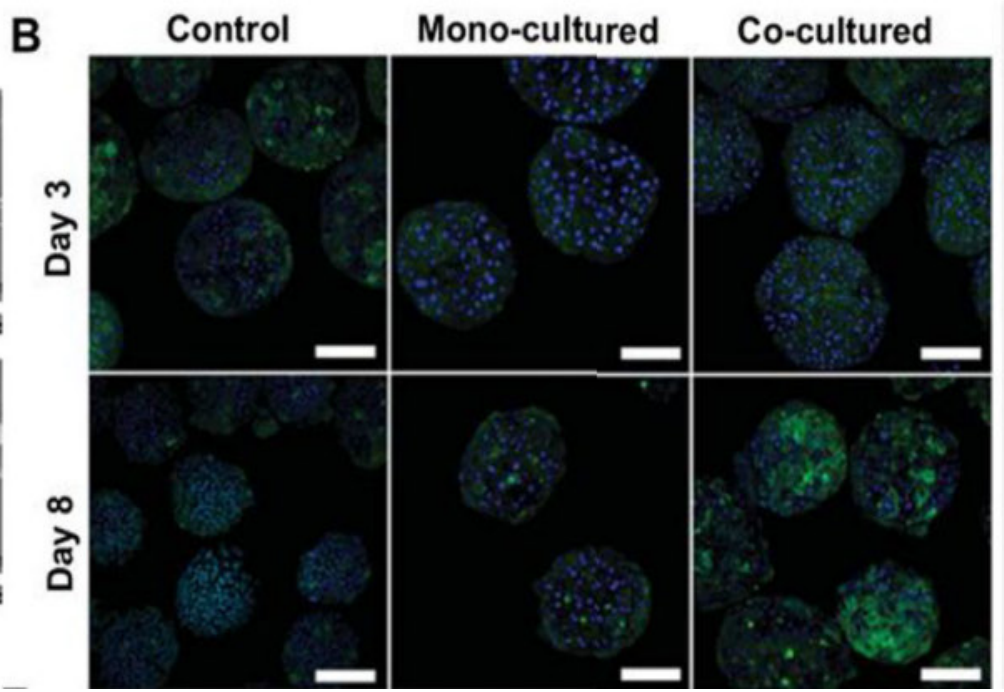
Hanging Droplets

Microfluidic Cell Trapping

a) Khetani, S. R. & Bhatia, S. N. at. Biotechnol. 26, 120–126 (2008).
b) Stevens, K. R. et al. Transl Med. 9, eaah5505 (2017).
c) Marga, F. et al. Biofabrication 4, 022001 (2012).
d) Rodenhizer, D. et al. Nat. Mater. 15, 227–234 (2016).
e) Frey, O., Misun, P. M., Fluri, D. A., Hengstler, J. G. & Hierlemann, A. Nat. Commun. 5, 4250 (2014).
f) Lai, B. F. L. et al. Adv. Funct. Mater. https://doi.org/10.1002/adfm.201703524 (2017).

Example: Liver-on-a-Chip…





Microwell array PDMS plate based liver-on-a-chip device.

Generated 3D spheroids on day 3 and 8.

Kostrzewski T, Cornforth T, Snow SA, Ouro-Gnao L, Rowe C, Large EM, et al. Three-dimensional perfused human in vitro model of non-alcoholic fatty liver disease. World J Gastroenterol. 2017 1 14;23(2):204–15.

Challenges

- Material selection
- Cellular fidelity
- Multiplexing and fluid handling
- Imaging and Sensing
- Validation and integration with existing drug development platforms.
- Scalable production, cost and market size.

Zhang B, Korolj A, Lai BFL, Radisic M. Advances in organ-on-a-chip engineering. *Nature Reviews Materials.* 2018;3(8):257-278.

Materials…

- PDMS (Silicone)
  - Elastic modulus of ~1-3 Mpa – compliant and deformable.
  - Easily moldable – 2-part mix, vacuum de-bubble and pour.
  - Sections can be plasma or ozone treated and “stacked” together allowing for complex microchannels.
  - Suitable for biomimetic ECM scaffolds.
  - Optically transparent, biocompatible and oxygen permeable.
  - Susceptible to medium evaporation, bubble formation and unwanted absorption of hydrophobic drugs/compounds.

Cellular Fidelity…

- Immortalized cell lines.
  - Serial changes affect genotype and phenotype
- ESCs (embryonic stem cells), and patient specific iPSCs (induced pluripotential cells).
  - More accurate modeling.
  - Differentiation may be accomplished within the device.
  - IPSCs can be expanded into heterogenous cell populations which can self-organize into organoids (fidelity of gene and protein expression, tissue morphology, and metabolic and physiological function.)
- Animal/human samples.
  - Can be a challenge to collect – especially myocardial cells.

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Fluid Handling…

- External pumping
  - Syringes, vacuum and peristaltic pumps.
- Integrated micropumps
  - e.g. pneumatic, electrostatic, magnetic and piezoelectric
- For control of nutrients, oxygen and waste removal.
- For circulation of signaling, metabolic and angiogenic factors.
- Integration of body-on-a-chip subunits.

Zhang B, Korolj A, Lai BFL, Radisic M. Advances in organ-on-a-chip engineering. *Nature Reviews Materials.* 2018;3(8):257-278.

Example Platform – Translational OOC…

https://youtu.be/jkq0BTILrq0

Body-on-a-Chip

1. Multiorgan systems on a chip.
2. Useful in predicting human response prior to clinical testing of a drug or as augmentation of clinical studies to test underlying mechanisms.
3. Modeling organ physiology allowing better understanding of underlying mechanisms of response to drugs and chemicals.
4. Animal models often do not predict human response effectively increasing the demand for more advanced model platforms.
5. Rate of development of these systems has been exponential.

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Sung JH, Wang YI, Narasimhan Sriram N, et al. Recent Advances in Body-on-a-Chip Systems. *Anal Chem*. Jan 2 2019;91(1):330-351. doi:10.1021/acs.analchem.8b05293
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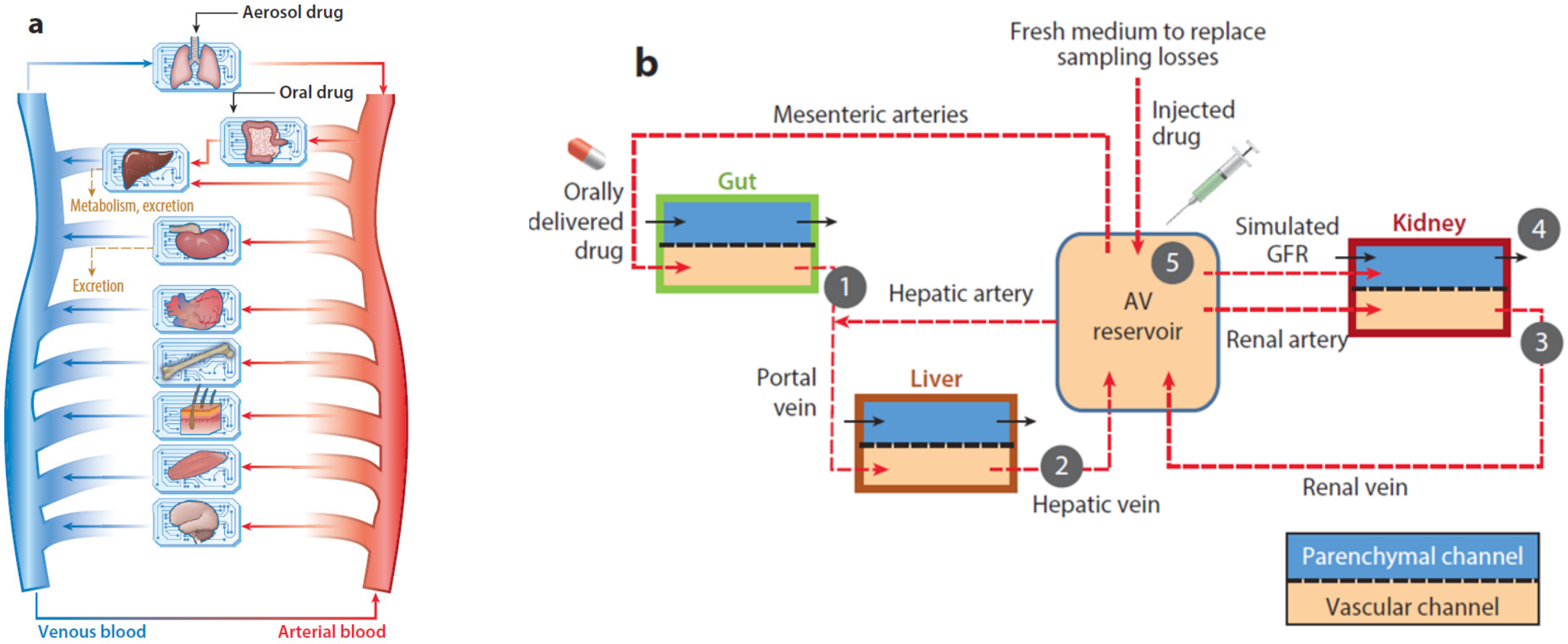
Body-on-a-Chip Concept…



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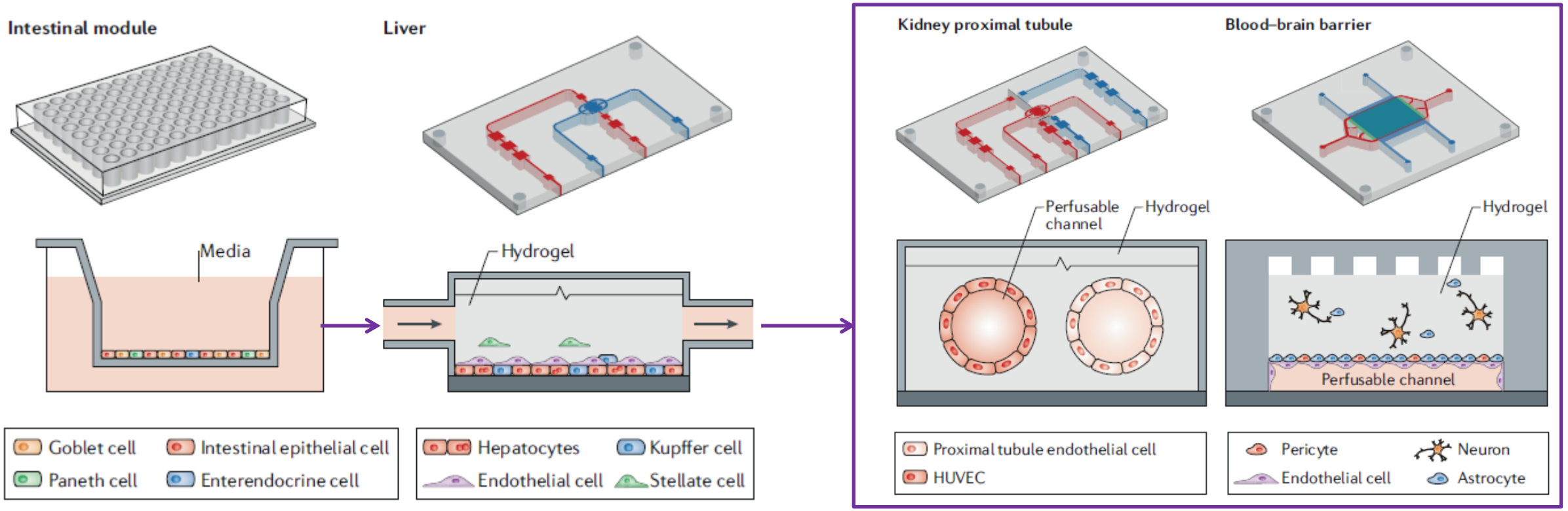
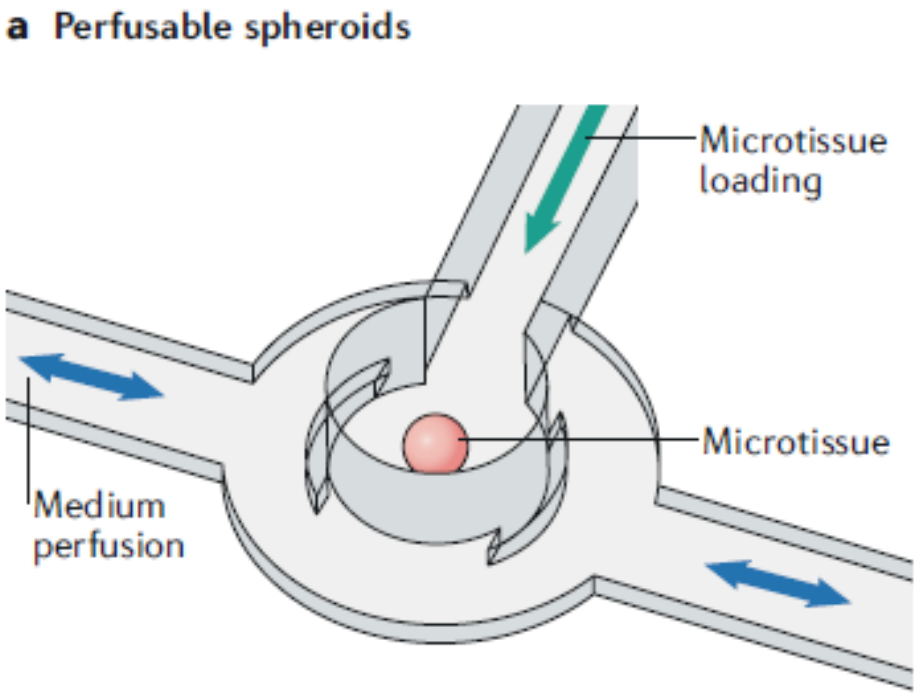
Prantil-Baun R, Novak R, Das D, Somayaji MR, Przekwas A, Ingber DE. Physiologically Based Pharmacokinetic and Pharmacodynamic Analysis Enabled by Microfluidically Linked Organs-on-Chips. In: Insel PA, ed. *Annual Review of Pharmacology and Toxicology, Vol 58.* Vol 58.2018:37-64.

Organ Coupling for Body-on-a-Chip…

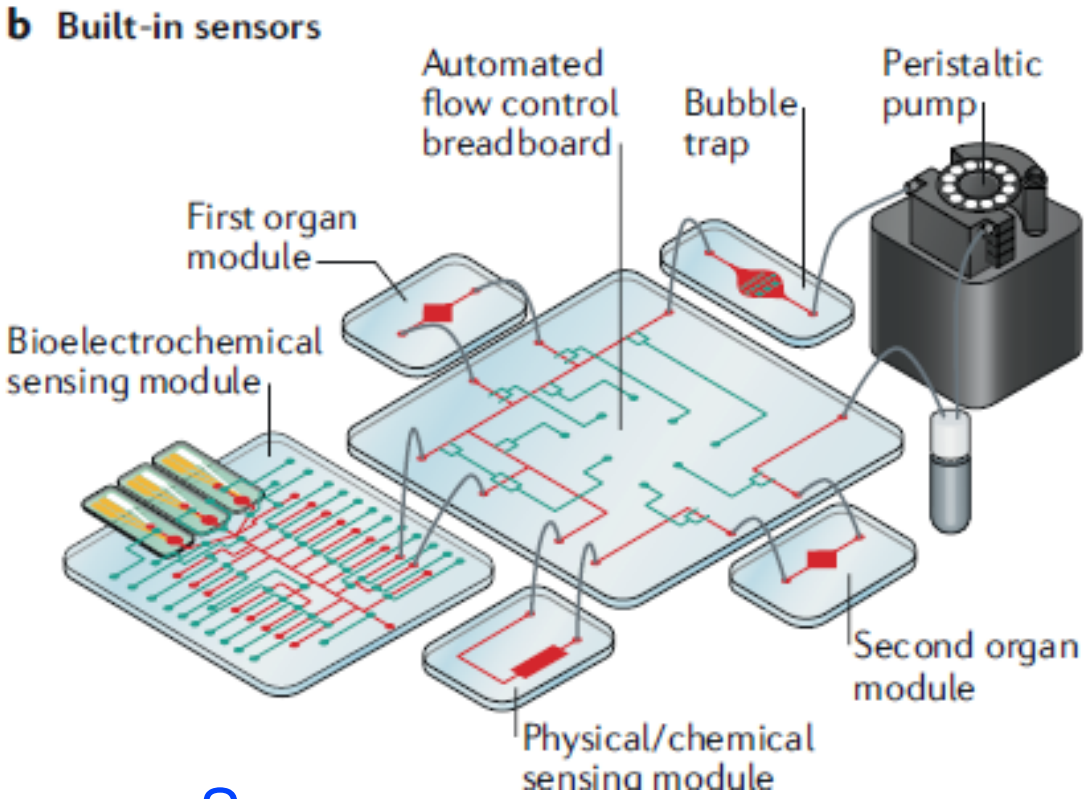


Vernetti, L. et al. Functional coupling of human microphysiology systems: intestine, liver, kidney proximal tubule, blood- brain barrier and skeletal muscle. Sci. Rep. 7, 42296 (2017).

Modeling Multi-Organ Interactions…



Spheroid



Senors

a) Kim, J.-Y. et al. 3D spherical micro-tissues and microfluidic technology for multi- tissue experiments and analysis. J. Biotechnol. 205, 24–35 (2015).
b) Zhang, Y. S. et al. Multisensor- integrated organs-on-chips platform for automated and continual in situ monitoring of organoid behaviors. Proc. Natl Acad. Sci. USA 114, E2293–E2302 (2017).

Imaging and Sensing

- Conventional optical and confocal fluorescence microscopes.
  - Post-printing bioreactors can now be followed in real-time while controlling the external environment.
- Microsensors can be made to monitor oxygen concentration, pH, glucose consumption.
- Antibody and aptamer-based sensing.
- Trans-epithelial electrical resistance (TEER)
  - Correlated with tissue health, drug delivery and tissue diffusion.

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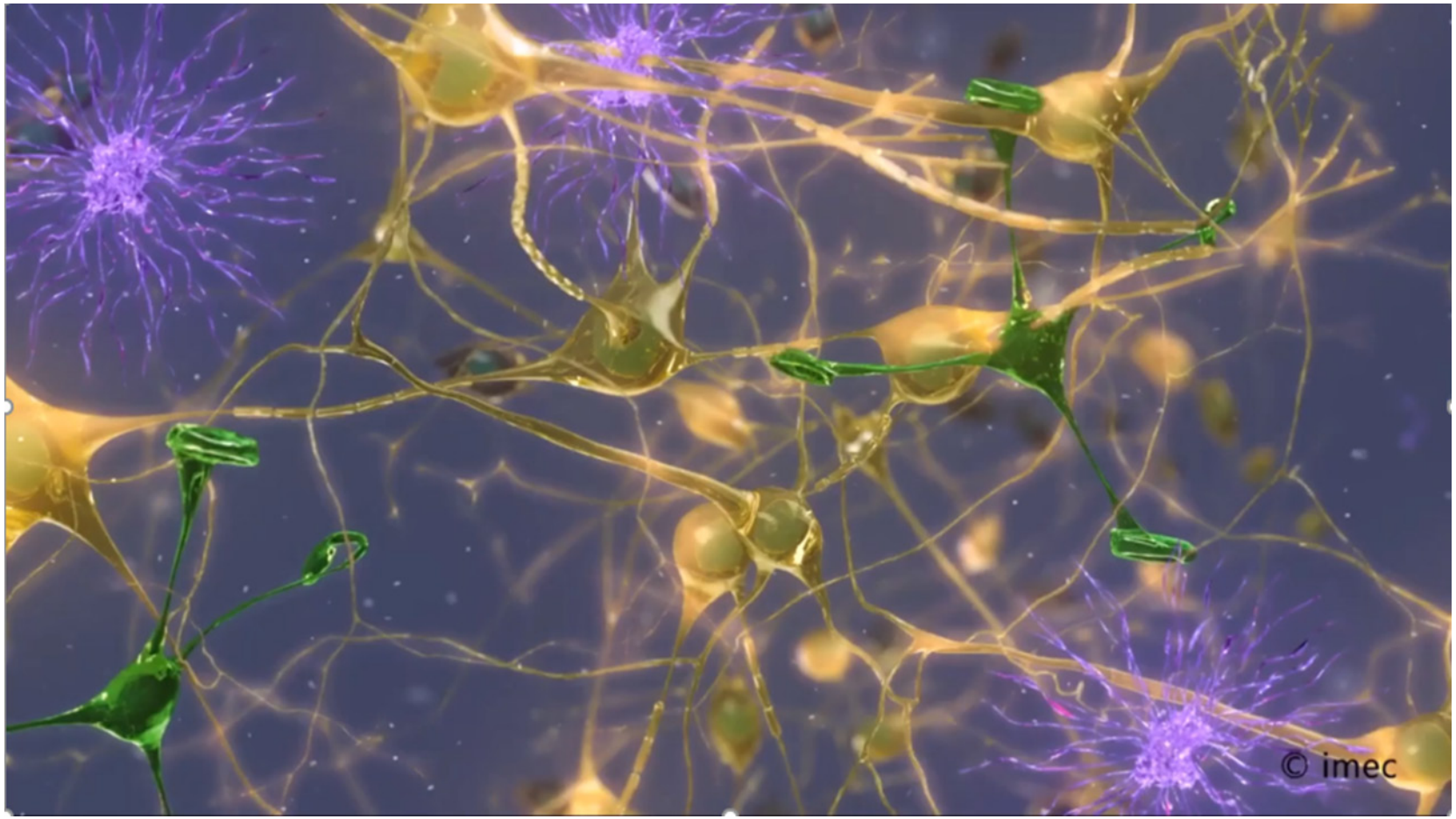
High Density Electrode for OOC Sensing…



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Summary

- Potential:
  - Drug discovery and cancer modeling.
  - ECM engineering for mimicking healthy and disease states.
  - Assessment of environmental chemical teratogenic effects.
- Biomimicking:
  - Multicellular vascular or epithelial interfaces of organs (ie. blood vessels, lung & gut).
  - Tissue-level organization of parenchymal cells (liver, heart, muscles tumors etc.)
  - Interaction of multiple organs (ie. drug absorption, distribution, metabolism, and elimination - *ADME*).

- Components
  - Geometric confinement and patterning.
  - Control of flow.
  - Environmental control.
  - Realtime monitoring of cellular activity and physiological data.
- Challenges
  - Material selection
  - Cellular fidelity
  - Multiplexing and fluid handling
- Body-on-a-Chip
- Imaging and Sensing

Addendum

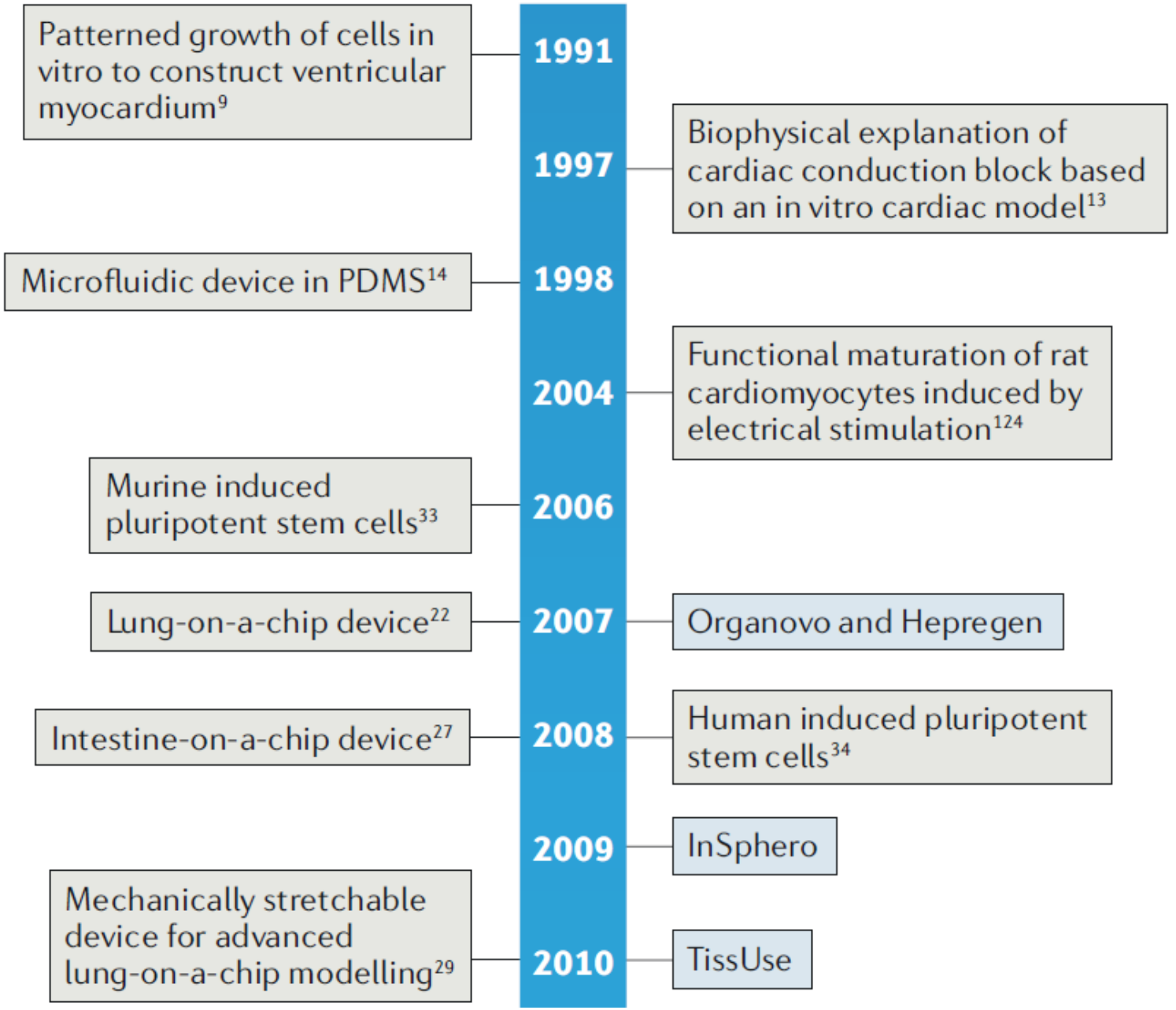
- Evolution of Organ-on-a-Chip Systems
- Drug Evaluation
- Validation
- Scalability
- OOC, Cell type, Condition & Drugs Tested

Evolution of Organ-on-a-Chip Systems

- Lab-on-a-chip systems emerged in the early 1990s, combining microfluidics technology, micro and nanofabrication methods, novel chemical sensors and analytical chemistries.
- The next step came with incorporating cells in the 2000s.
  - Movement from *in vitro* to *in vivo-like* environments.
  - Recognition of the ECM role in signalling, cell growth, adhesion, mechanical environment, among others.
  - Need to supply nutrients and oxygen, and remove waste products. Desire to move from diffusion only systems to creating *vasculature*.

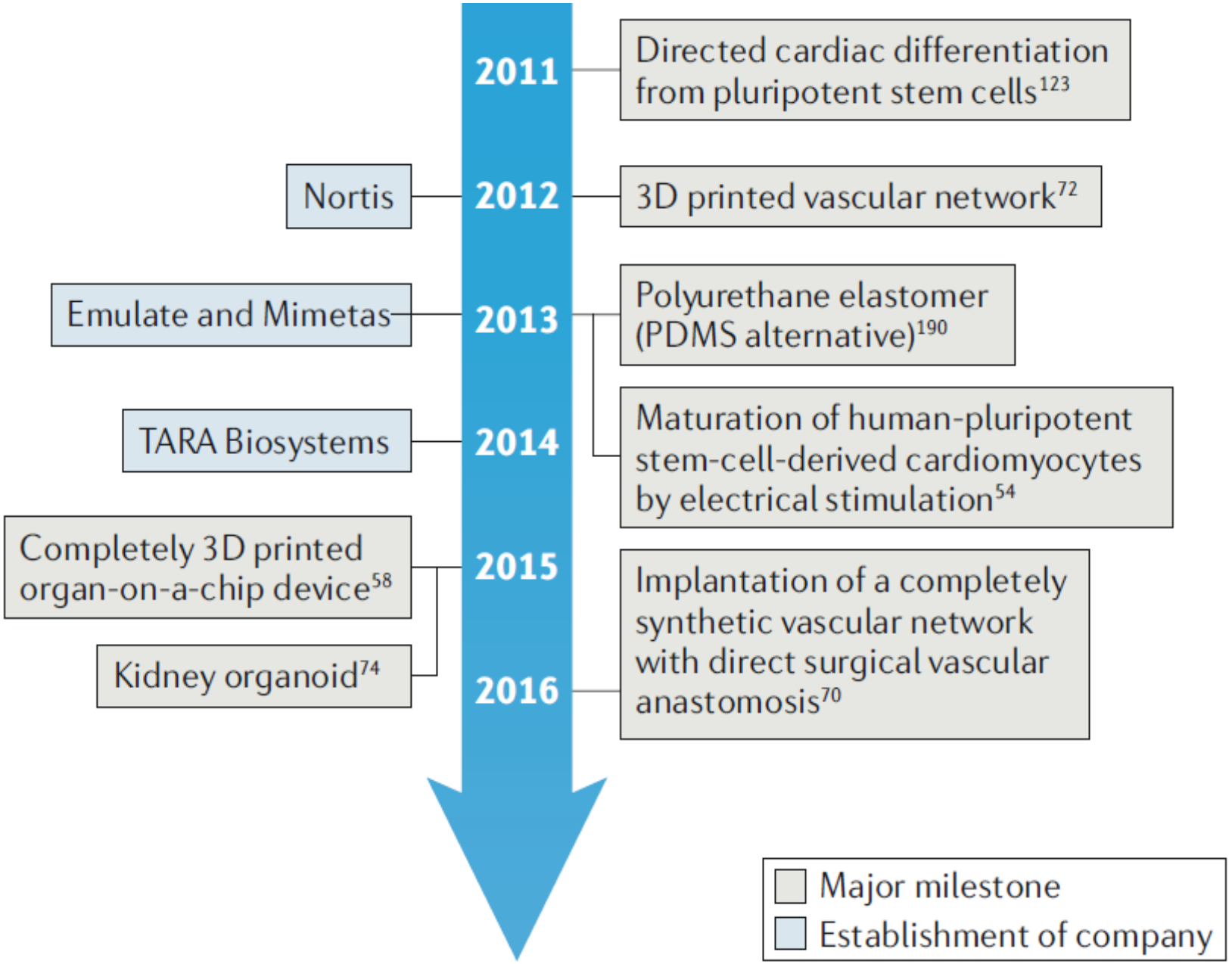
Kimura H, Sakai Y, Fujii T. Organ/body-on-a-chip based on microfluidic technology for drug discovery. *Drug Metabolism and Pharmacokinetics.* 2018;33(1):43-48.

Milestones



Companies Formed

Zhang B, Korolj A, Lai BFL, Radisic M. Advances in organ-on-a-chip engineering. *Nature Reviews Materials.* 2018;3(8):257-278.

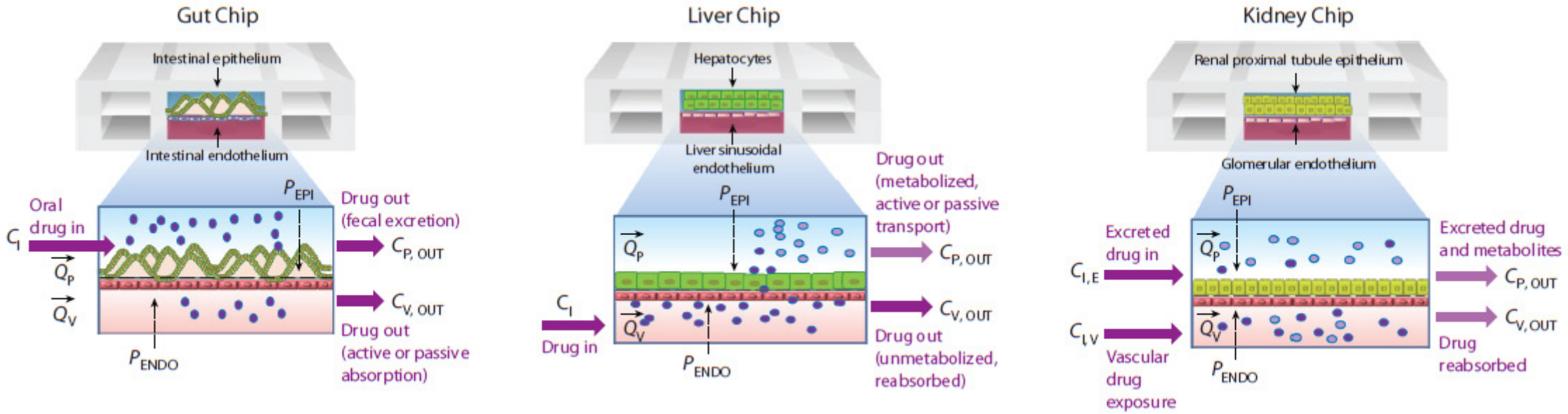

2011
Directed cardiac differentiation from pluripotent stem cells[123]
Nortis
2012
3D printed vascular network[72]
Emulate and Mimetas
2013
Polyurethane elastomer (PDMS alternative)[190]
TARA Biosystems
2014
Maturation of human-pluripotent stem-cell-derived cardiomyocytes by electrical stimulation[54]
Completely 3D printed organ-on-a-chip device[58]
2015
Implantation of a completely synthetic vascular network with direct surgical vascular anastomosis[70]
Kidney organoid[74]
2016
Major milestone
Establishment of company

Drug Evaluation with Organ Chips

- Microfluidic cell culture devices and vascularized Organ Chips offer a potentially exciting new approach to predict PK/PD properties in vitro.
- Vascularized microfluidic models that reconstitute physiological features and multicellular phenotypes are superior to typical cell lines, plate-based assays, and single-channel microfluidic devices.
- Computational abilities offer the capability to run more physiological experiments and to extrapolate in vivo predictions of drug behaviors in humans from in vitro data obtained with linked vascularized organs-on-a-chip.

Prantil-Baun R, Novak R, Das D, Somayaji MR, Przekwas A, Ingber DE. Physiologically Based Pharmacokinetic and Pharmacodynamic Analysis Enabled by Microfluidically Linked Organs-on-Chips. In: Insel PA, ed. *Annual Review of Pharmacology and Toxicology, Vol 58.* Vol 58.2018:37-64.

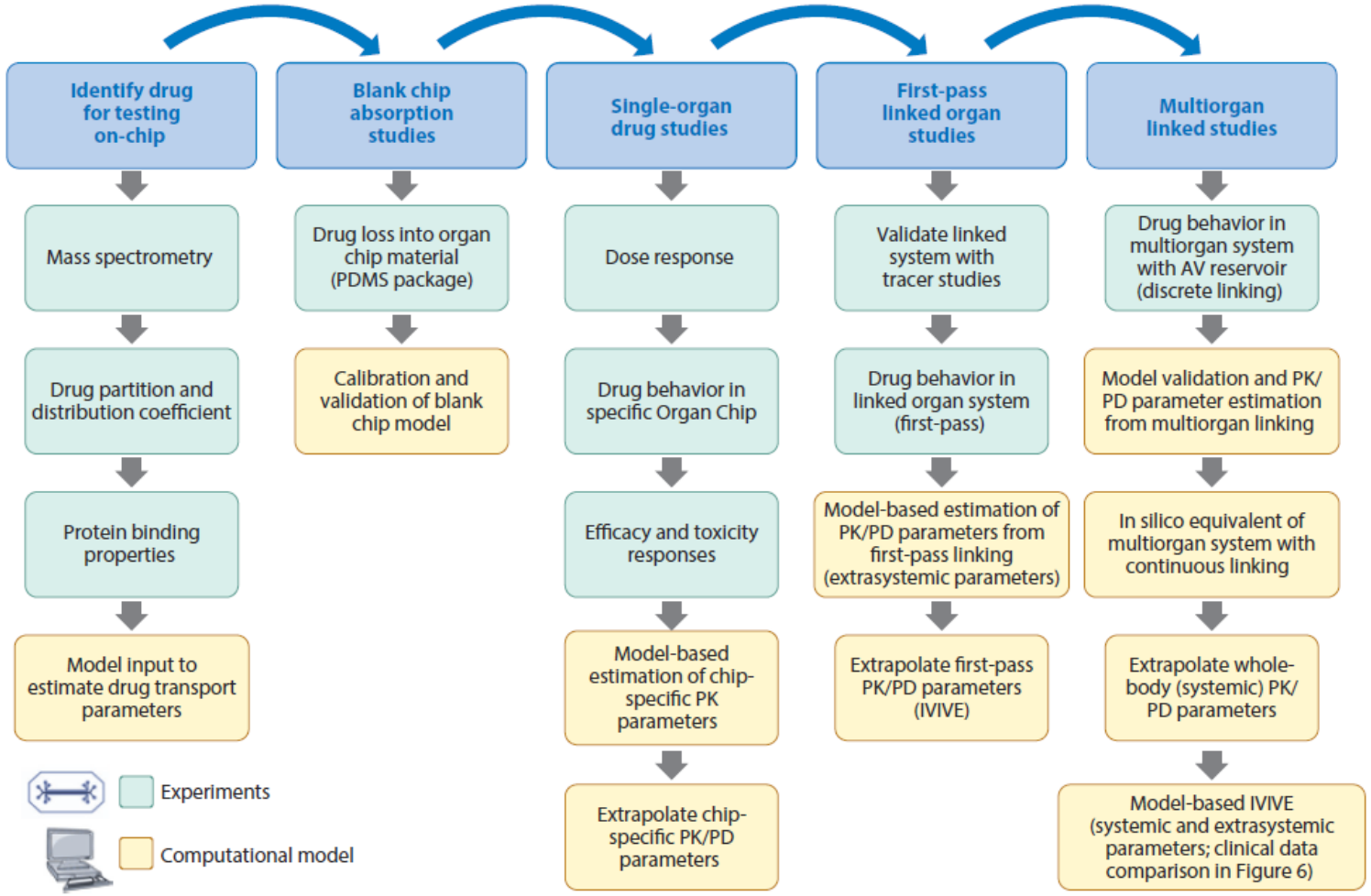
Individual Organs-on-a-Chip…



Schematics of how pharmacokinetic (PK) parameters are measured in Human Gut, Liver, and Kidney Chips. All three chips are lined with organ-specific epithelium(upper channel) and vascular endothelium (lower channel) on opposite sides of a porous, matrix coated membrane in the central two channels of the device.

Prantil-Baun R, Novak R, Das D, Somayaji MR, Przekwas A, Ingber DE. Physiologically Based Pharmacokinetic and Pharmacodynamic Analysis Enabled by Microfluidically Linked Organs-on-Chips. In: Insel PA, ed. *Annual Review of Pharmacology and Toxicology, Vol 58.* Vol 58.2018:37-64.

Work Flow to Evaluate a Drug…

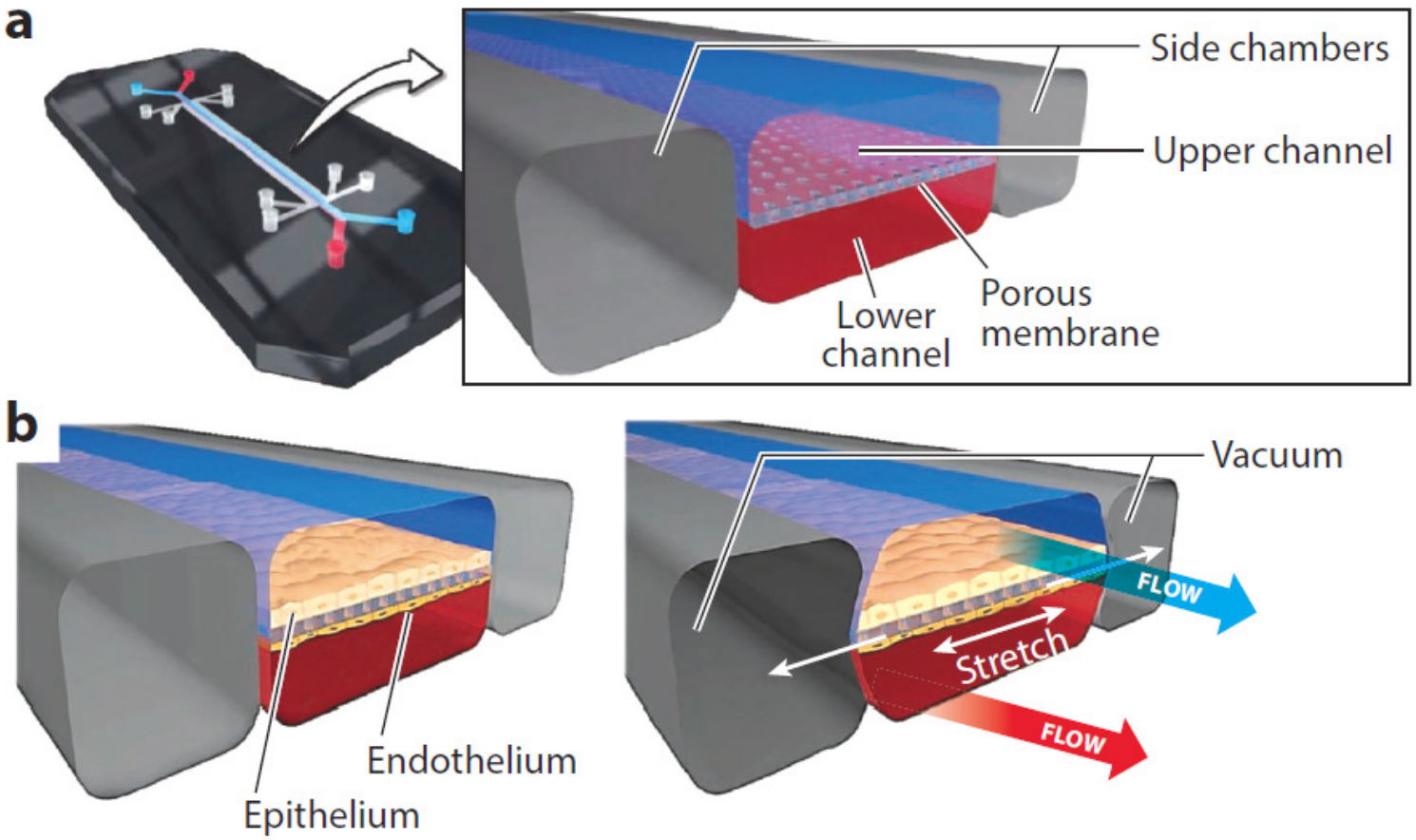


Work flow to evaluate a drug in linked organ studies using a Human Body–on–Chips and to extrapolate pharmacokinetic /pharmacodynamic (PK/PD) parameters.

Steven S. Saliterman

Prantil-Baun R, Novak R, Das D, Somayaji MR, Przekwas A, Ingber DE. Physiologically Based Pharmacokinetic and Pharmacodynamic Analysis Enabled by Microfluidically Linked Organs-on-Chips. In: Insel PA, ed. *Annual Review of Pharmacology and Toxicology, Vol 58.* Vol 58.2018:37-64.

Vascularized Organ-on-a-Chip…



Prantil-Baun R, Novak R, Das D, Somayaji MR, Przekwas A, Ingber DE. Physiologically Based Pharmacokinetic and Pharmacodynamic Analysis Enabled by Microfluidically Linked Organs-on-Chips. In: Insel PA, ed. *Annual Review of Pharmacology and Toxicology, Vol 58.* Vol 58.2018:37-64.

Validation

- Validation will ensure that the biological functions produced on the chip are representative of the native tissue, with an understanding of any lack of similarity.
- Translational research is dependent on collaboration between researcher and industry.
- FDA and other regulatory collaboration with industry is necessary for standards – and selecting suitable physiological hallmarks, for validation of organ-on-a chip devices.

Zhang B, Korolj A, Lai BFL, Radisic M. Advances in organ-on-a-chip engineering. *Nature Reviews Materials.* 2018;3(8):257-278.

Scalability

- Research-oriented soft lithography techniques are slow, expensive, are typically manually completed with multiple process steps and low yield, and lack reproducibility.
  - 3D printing/bioprinting may be useful –especially for complex microarchitectures and embedded sensors.
  - Preservation of printed cell/tissues within devices (device shelf-life) is a challenge.

Zhang B, Korolj A, Lai BFL, Radisic M. Advances in organ-on-a-chip engineering. *Nature Reviews Materials.* 2018;3(8):257-278.

OOC, Cell type, Condition & Drugs Tested

OOC	Cell types used	Target disease or condition	Drugs tested	Functionality tested	Ref.
Lung on a chip	Cell line	Lung Cancer (NSCLC)	Tyrosine kinase inhibitor	Mechanical Strain, transfer across epithelial-endothelial tissue-tissue interface	(122)
Liver on a chip	Primary hepatocytes	Non-alcoholic fatty liver disease (NAFLD)	Pioglitazone, metformin	Oxygen gradient, Cell phenotyping, fat accumulation, metabolic capacity	(59)
	Primary hepatocytes	Potential drug toxic effects	Acetaminophen (APAP)	Configuration, arrangement of capillary layers, inflammatory reaction towards enhanced cellular stress, expression of genes	(60)
	Primary hepatocytes	Drug-induced liver injury (DILI)	Troglitazone	Efflux media collection, compatibility for microfluidic coupling, clearance rates for drugs	(123)
	Cell line (HepG2/C3A)	Mitochondrial dysfunction	Troglitazone, rotenone	3D cell aggregates, real-time oxygen measurement, mitochondrial Stress	(61)

Jodat YA, Kang MG, Kiaee K, et al. Human-Derived Organ-on-a-Chip for Personalized Drug Development. *Current pharmaceutical design*. 2018;24(45):5471-5486. doi:10.2174/1381612825666190308150055

Kidney on a chip	hiPSCs	Albuminuria	Adriamycin	Cyclic mechanical strain, urinary filtrate, regulated vacuum, kidney glomerular capillary wall	(67)
	Primary cell	CysA-induced damage	Cyclosporine A	Renal proximal tubule (PT) composed of a perfusable open lumen that possesses a programmable architecture	(68)
	Primary cell	Chronic kidney disease, urothelial cancer	Aristolochic acid I	Organ-organ interactions	(69)
Gut on a chip	Cell line	Drug absorption	SN-38 (7-ethyl-10-hydroxycamptothecin)	Barrier function, microvilli expression, permeability coefficient	(124)
	Cell line	Gut radiation injury	DMOG (dimethyl oxaloylglycin)	Villus differentiation, paracellular permeability, radio-protective effects of drug	(72)
	hiPSCs	Biologically responsive to exogenous stimuli	Tumor necrosis factor-α, IFN-γ	Epithelial-immune cell interactions, permeability	(125)

Jodat YA, Kang MG, Kiaee K, et al. Human-Derived Organ-on-a-Chip for Personalized Drug Development. *Current pharmaceutical design*. 2018;24(45):5471-5486. doi:10.2174/1381612825666190308150055

CNS and PNS on a chip	Human neural progenitor cell line	Molecular toxicology	Acetaminophen, 5-fluorouracil, retinoic acid, doxorubicin, pitavastatin	Various cell states, protein expression	(75)
	hiPSC-derived neurons	Familial Alzheimer's disease	β-secretase inhibitor	Fluidic isolation, separation of axons from the soma	(76)
	Primary cell, hiPSC	Motor neuron disease	Motor neuron progenitor	Effect of microvascular network perfusion on neural activity	(126)

Jodat YA, Kang MG, Kiaee K, et al. Human-Derived Organ-on-a-Chip for Personalized Drug Development. *Current pharmaceutical design*. 2018;24(45):5471-5486. doi:10.2174/1381612825666190308150055