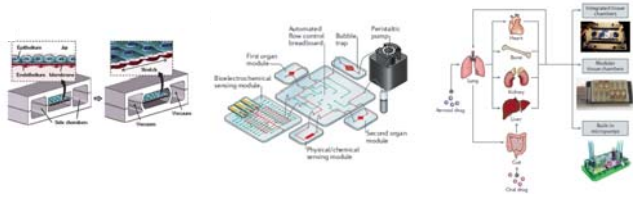


Organ-on-a-Chip (OOCs)

Prof. Steven S. Satterman, <http://satterman.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!

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- 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



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Cost of Drug Development

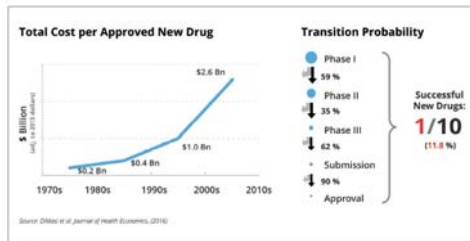


Figure 1 Costs of drug development have risen while overall probability of regulatory approval has reduced. Image taken from DiMasi JA et al. J Health Econ. 2016;47:20-33

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Organ-on-a-Chip Research



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<https://youtu.be/5l0QVeFbS0c>

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, Fuji T. Organbody-on-a-chip based on microfluidic technology for drug discovery. *Drug Metabolism and Pharmacokinetics*. 2018;33(1):43-48.
Zhang B, Korolij A, Lai BFL, Radtke M. Advances in organ-on-a-chip engineering. *Nature Reviews Materials*. 2018;3(8):257-278.

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- 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.

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Cell Cycle for Personalized Medicine...



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Jodati 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:10.2174/1381612825668190308190055

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).

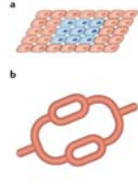
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Zhang B, Korolj A, Lai BFL, Radtke M. Advances in organ-on-a-chip engineering. *Nature Reviews Materials*. 2018;3(8):257-278.

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:

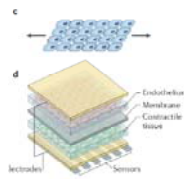
- Fluid inlets and outlets.
- Physiological cell to liquid ratio.

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Maoz, B. M. et al. Organ-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 O₂, CO₂, 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.

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Maoz, B. M. et al. Organ-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.

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

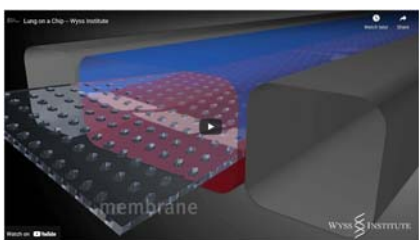
What's Required for Organ-Specific Structures?

- **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.

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

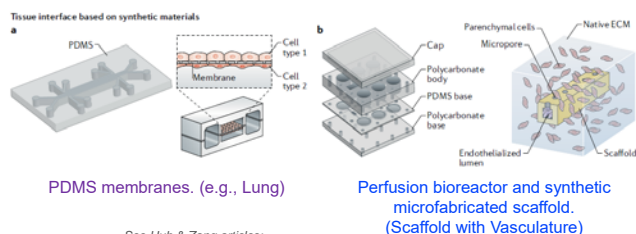
Lung-on-a-Chip...



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<https://youtu.be/52IL9gemyDw>

Reproducing the Tissue Barrier Function...

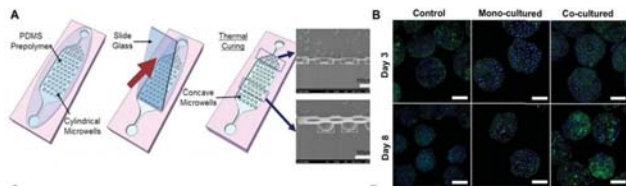


See Huh & Zang articles:

a) Huh, D. et al. Reconstituting organ-level lung functions in 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).

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Example: Liver-on-a-Chip...



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

Generated 3D spheroids on day 3 and 8.

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Kostrzewski T, Cornforth T, Snow SA, Ours-Groo L, Rowe C, Large EM, et al. Three-dimensional perfused human in vitro model of non-alcoholic fatty liver disease. *World J Gastroenterol*. 2017;11:1423-204-15.

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.

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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.

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Cellular Fidelity...

- **Immortalized cell lines.**
 - Serial changes affect genotype and phenotype
- **ESCs (embryonic stem cells), and patient specific iPSCs (induced pluripotent 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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Zhang B, Korolj A, Lai BFL, Radisic M. Advances in organ-on-a-chip engineering. *Nature Reviews Materials*. 2018;3(8):257-278.

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.**

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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...



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<https://youtu.be/jkq0BTLrq0>

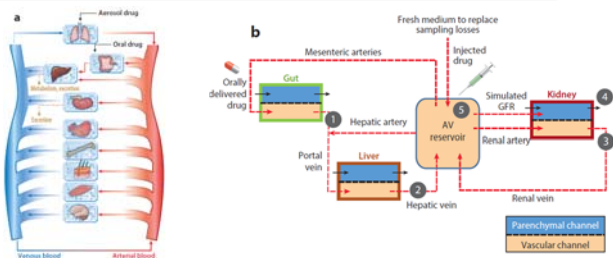
Body-on-a-Chip

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

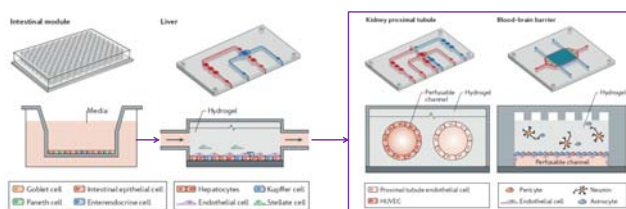
Body-on-a-Chip Concept...



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Prasidi Ganu R, Novak R, Das D, Somayaji MR, Prasanna 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.

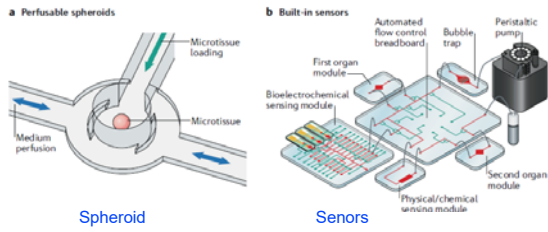
Organ Coupling for Body-on-a-Chip...



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Verneth L, et al. Functional coupling of human microphysiology systems: intestine, liver, kidney proximal tubule, blood-brain barrier and skeletal muscle. *Sci. Rep.* 7, 42266 (2017).

Modeling Multi-Organ Interactions...



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 organ-on-chips platform for automated and continual in situ monitoring of organoid behaviors. *Proc. Natl. Acad. Sci. USA* 114, E2293–E2302 (2017).

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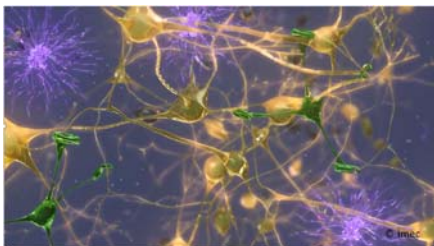
Imaging and Sensing

- **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.

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

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High Density Electrode for OOC Sensing...



<https://youtu.be/FcV0vCXyF4k>

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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*).

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- 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

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Addendum

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

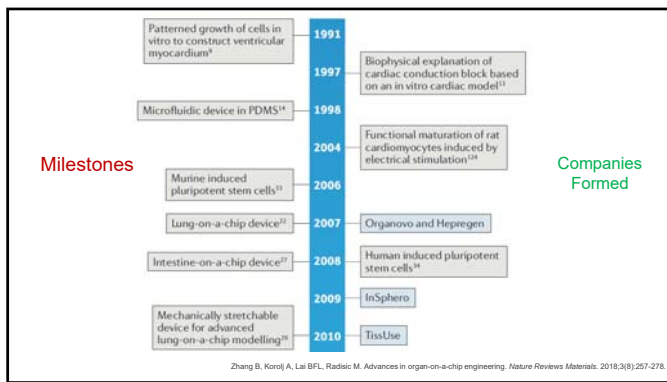
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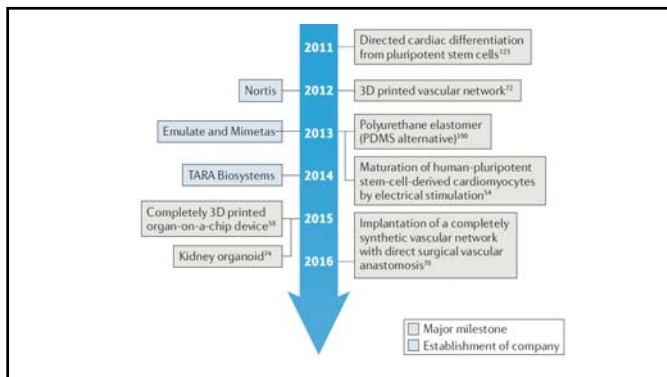
Evolution of Organ-on-a-Chip Systems

- 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*.

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



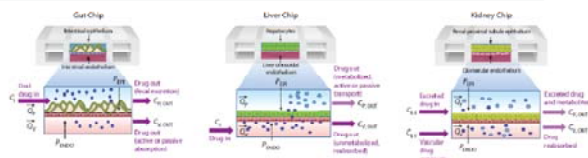


Drug Evaluation with Organ Chips

- 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.

Steven S. Sallerman, 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: *Insil PA, ed. Annual Review of Pharmacology and Toxicology*, Vol 58. Vol 58. 2018:37-64.

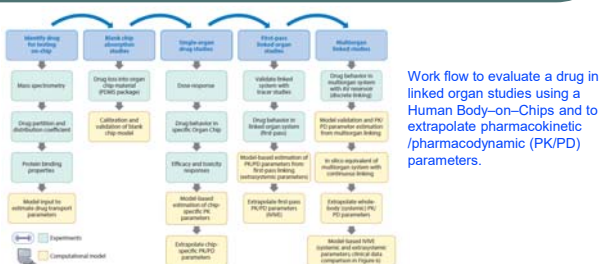
Individual Organs-on-a-Chip...



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.

Steven S. Sallerman, 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: *Insil PA, ed. Annual Review of Pharmacology and Toxicology*, Vol 58. Vol 58. 2018:37-64.

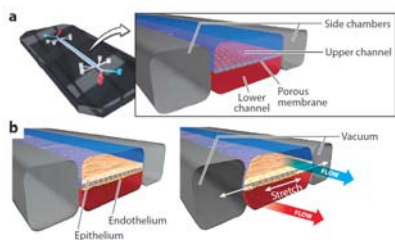
Work Flow to Evaluate a Drug...



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. Sallerman, 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: *Insil PA, ed. Annual Review of Pharmacology and Toxicology*, Vol 58. Vol 58. 2018:37-64.

Vascularized Organ-on-a-Chip...



Prantl-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.

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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.

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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.

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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 inhibitors	Mechanical Stress, vascular access, epithelial endothelial tissue-tissue interface	(122)
	Primary hepatocytes	Non-alcoholic fatty liver disease (NAFLD)	Phlegbotonin, sorafenib	Oxygen gradient, Cell permeability, metabolic capacity	(86)
Liver on a chip	Primary hepatocytes	Potential drug toxic effects	Acetaminophen (APAP)	Cell apoptosis, arrangement of capillary lumen, inflammatory reaction, necrosis, cytokine/cellular stress, expression of genes	(60)
	Primary hepatocytes	Drug induced liver injury (DILI)	Tegaserod	Efflux molecule collection, compatibility for microfluidic coupling, clearance rates for drugs	(123)
	Cell line (HepG2/C3A)	Mitochondrial dysfunction	Etoposide, rotenone	2D cell aggregates, real time oxygen measurement, mitochondrial stress	(61)

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Jodai YA, Kang MG, Kjaee K, et al. Human-Derived Organ-on-a-Chip for Personalized Drug Development. *Current pharmaceutical design*. 2018;24(45):5471-5486. doi:10.2174/1381612825668190308150055

Kidney on a chip	hiPSCs	Alzheimers	Achimycin	Cyclic nucleotid stress, sensory EDRs, regulated necrosis, kidney phosphate signaling, cell	(67)
	Primary cell	CysA induced damage	Cyclosporin A	Renal peritubular tubule (PT) composed of a peritubular open lumen that provides a programmable microclimate	(68)
	Primary cell	Chemical kidney disease, metabolic cancer	Acetothic acid I	Organ organ interactions	(69)
Gut on a chip	Cell line	Drug absorption	SN-38 (7-ethyl-10-hydroxycaoptothecin)	Barrier function, mucin-III expression, permeability coefficients	(124)
	Cell line	Gut reduction injury	DMOO (dimethyl oxidolipic acid)	Villus differentiation, permeability, redox-protection effects of drug	(72)
	hiPSCs	Biologically responsive to exogenous stimuli	Tumor necrosis factor α , BDNF	Epithelial immune cell interactions, permeability	(125)

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Jodai YA, Kang MG, Kjaee K, et al. Human-Derived Organ-on-a-Chip for Personalized Drug Development. *Current pharmaceutical design*. 2018;24(45):5471-5486. doi:10.2174/1381612825668190308150055

CNS and PNS on a chip	Human neural progenitor cell line	Molecular toxicology	Acetaminophen, 5-fluorouracil, retinoic acid, doxorubicin, piperazine	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)

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Jodai YA, Kang MG, Kjaee K, et al. Human-Derived Organ-on-a-Chip for Personalized Drug Development. *Current pharmaceutical design*. 2018;24(45):5471-5486. doi:10.2174/1381612825668190308150055