CHEM-E8135 Microfluidics and BioMEMS

Organs-on-Chips

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Intended learning outcomes

The student understand the definition, concept and purpose of organ-on-chips.

The student can name several types of organ on chips and describe their basic idea.

The student knows the basics of organ-on-chip design

Outline

1. Introduction
2. Lung-on-a-chip

-we learn the concept of mimicing a feature with this example

3. CNS, Kidney and Gut models

-short overview of other types to see similarities and differences

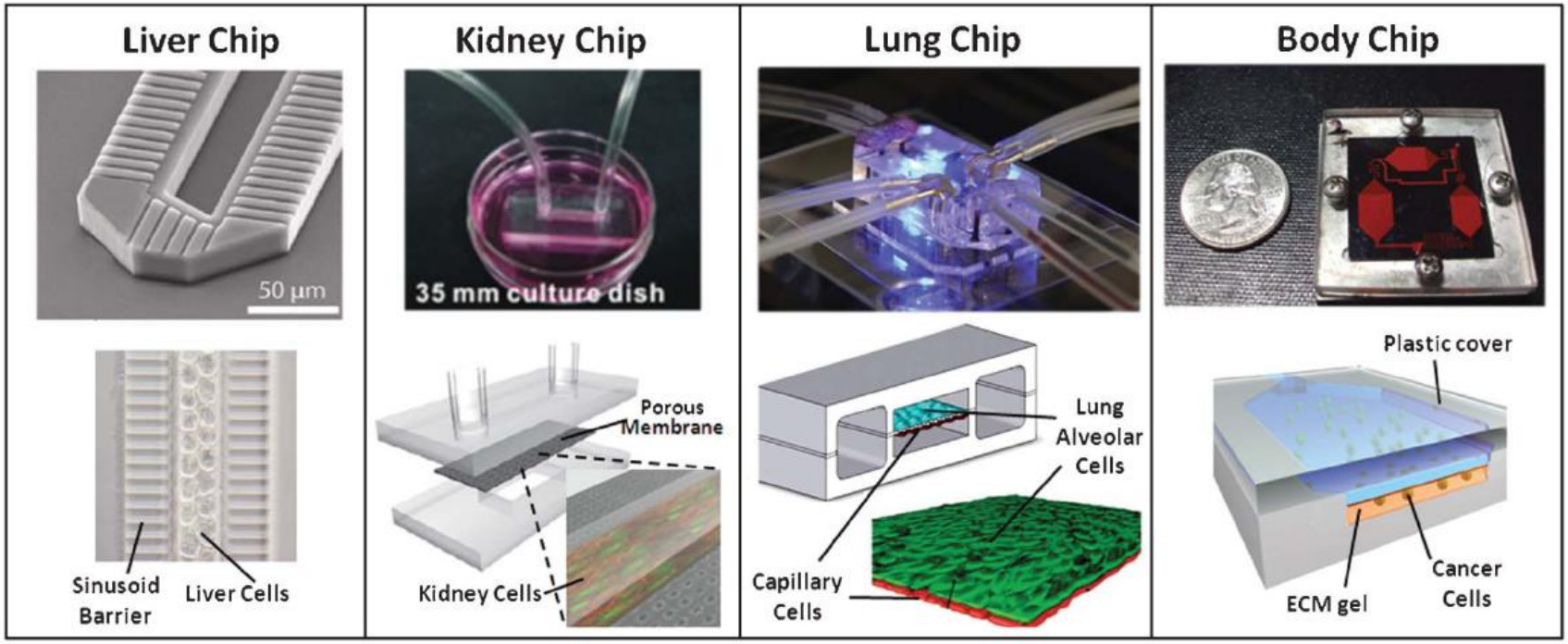
4. A task for students, create definition for OoC

-What is organ-on-a-chip exactly?

5. A brief overview of materials, cell types, fabrication, commercialization

Organ-on-chip, what are they?

- Miniaturized, microchip based models of organs
- Current status: proof-of-concept studies, basic biomedical research
- In future, a solution to rising costs of pharmaceutical development?



Why organs-on-a-chip?

- Basic research: Possibility for experimentation at a level intermediate to cell culture models and animal models.
- Pharmaceutical industry: Need for more efficient screening

- **Animal models:**

+ Direct experimentation on in vivo conditions
- Ethical issues, time and cost. Biological complexity can be overwhelming.

- **Cell culture models:**

+ Simplicity
- Lack of architecture
- Far removed from in vivo conditions

- **Organ-on-a-chip models:**

+ A middle ground between cell culture and animal models?
+ Can utilize human cells also
- Not a mature field yet
- Largely undemonstrated for actual biological and biomedical research.

Modeling of organ functions

Organ-on-a-chip should reproduce, mimic or approximate some aspect ofthe target organ that is missing on a petri dish. For example:

1. **Architecture!**
2. **Movement (fluid flow, stretching / compression)**
3. Chemical environment (pH, concentrations of proteins, oxygen etc.)
4. Physical environment (soft/hard, air/liquid)

Levels of modeling:

1 to 1 recreation of biological processes on chip. The ideal, but unrealistic.

One or several aspects of the organ mimicked, some other aspects are non-physiological. The typical Organ-on-a-chip.

No mimicking of any aspect of organ beyond a well plate. This is not an Organ-on-a-chip but it can still be extremely useful for other purposes.

Complexity increases:
-more challenging to make and operate.
-less biological artifacts
-more/less engineering artifacts

Example: Lung model

Reconstituting Organ-Level Lung Functions on a Chip
Dongeun Huh, Benjamin D. Matthews, Akiko Mammoto, Martín Montoya-Zavala, Hong Yuan Hsin, Donald E. Ingber
25 JUNE 2010 VOL 328 SCIENCE

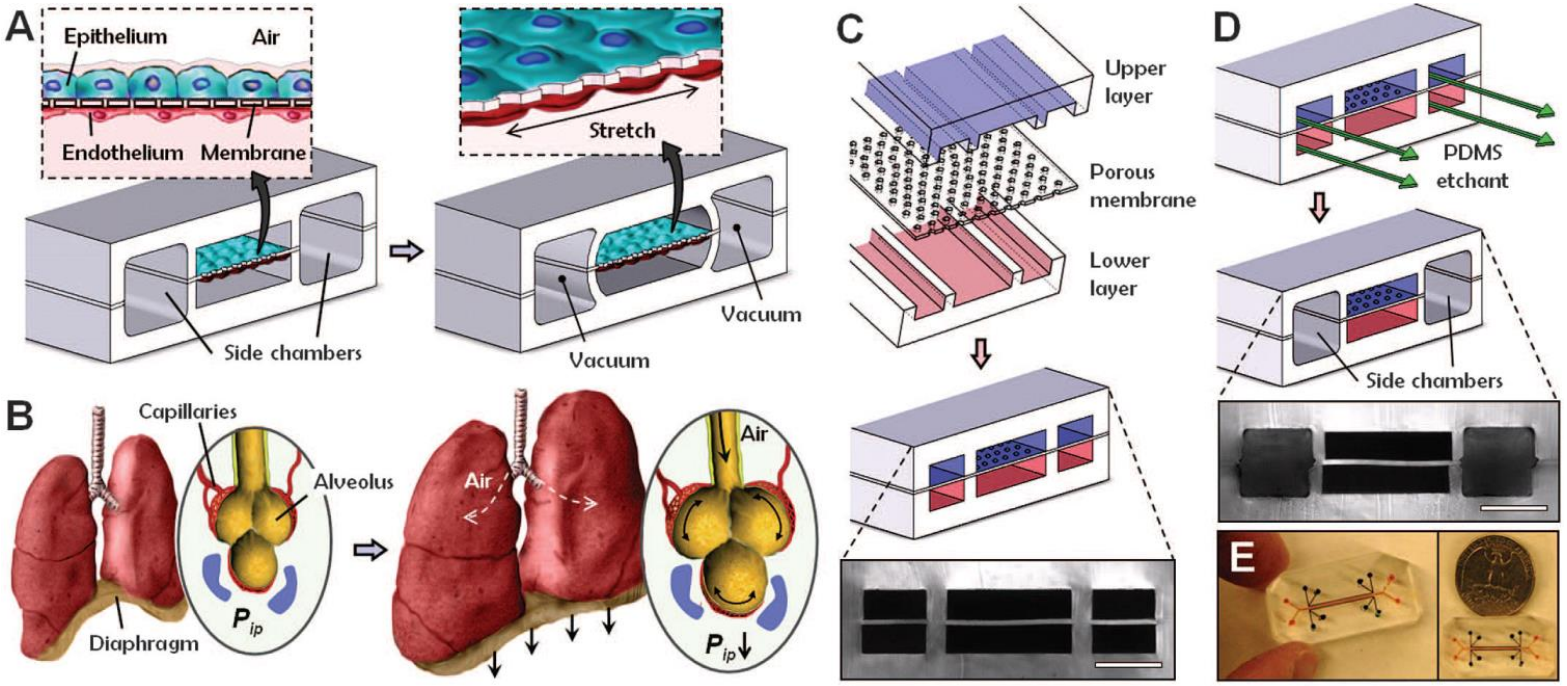
#1 "**Reconstitutes** the critical functional alveolar-capillary interface of the human lung"

#2 "**Reproduces** complex integrated organ-level responses to bacteria and Inflammatory cytokines"

#3 "**Cyclic mechanical strain accentuates** toxic and inflammatory responses of the lung to silica nanoparticles"

#4 "**Mechanical strain also enhances** epithelial and endothelial uptake of nanoparticulates and stimulates"

Technical overview



- 2 microchannel layers and one porous (10 µm) layer of PDMS, bonded together
- The membrane coated with ECM proteins (fibronectin, collagen) for cell adesion.
- One side of the membrane seeded with human alveolar epithelial cells. The other side with human pulmonary microvascular cells.
- Epithelial and endothelial side individually addressable. Air/water.
- Breathing motion by applying vacuum to the side channels. 10%, 0.2Hz

What is modeled?

- Two distinct types of cells organized into epithelium/endothelium

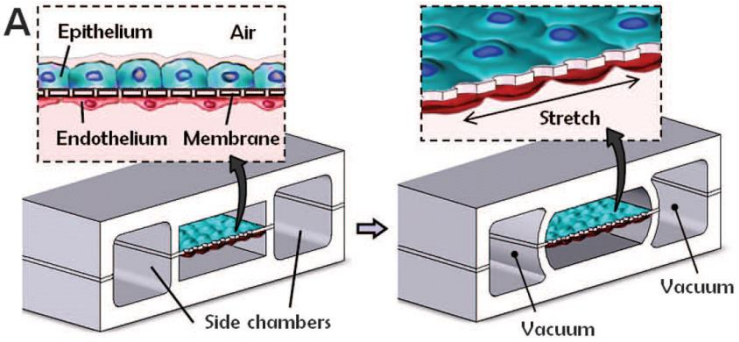
= alveolar capillary interface?

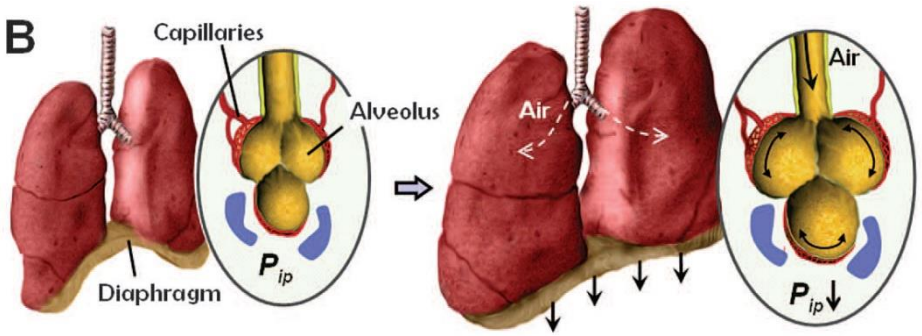
- Repeated stretching and stretch relaxation

=breathing motion?

- Alveolar capillary interface + breathing motion + air and liquid channels

=lung?



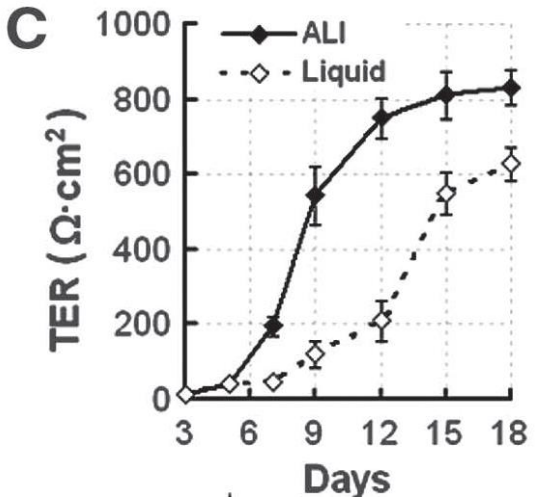


Alveolar capillary interface

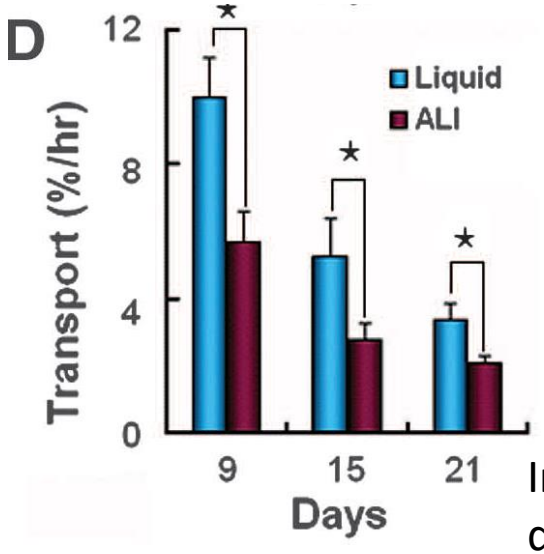
- Intact monolayers of cells linked by epithelial and endothelial junctional proteins.
- Epithelial side produces surfactants to stay moist against air
- Electrical resistance increased and protein transport decreased on the membrane formed at air-liquid interface as compared to fully liquid submerged.
- Quantitative or qualitative? 2.1%/h permeability of albumin on chip, 1-2%/h in vivo

ALI = Liquid/air

Liquid = Liquid/liquid



Increased electrical barrier properties



Increased protein diffusion barrier properties

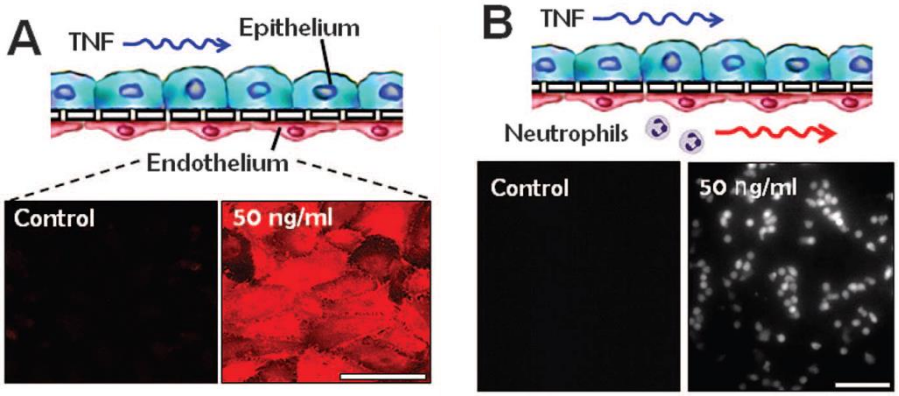
Organ level inflammation response

- Irritation (bacteria, particles, etc) of the epithelial side

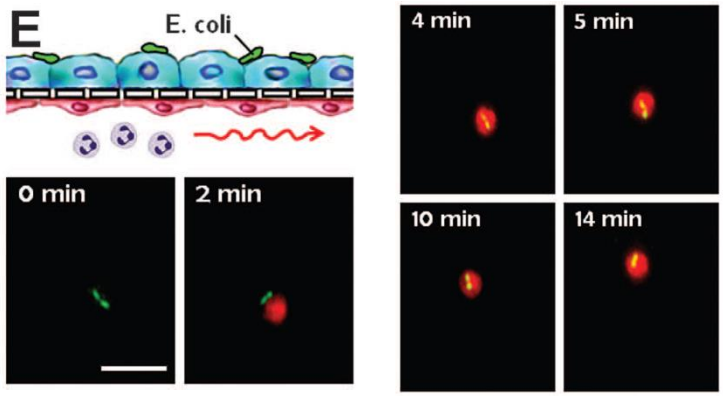
-> Upregulation of ICAM-1 (inter cellular adhesion molecule 1)

-> Recruitment of neutrophils from the endothelial side

Breathing chip (10%, 0.2 Hz) vs static chip as Control



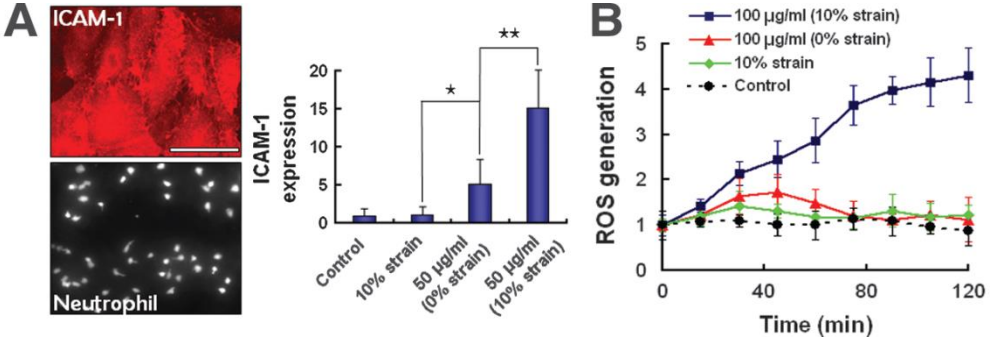
Tumor necrosis factor introduced to alveolar compartment
Endothelium produces ICAM-1
ICAM-1 recruits neutrophils from the endothelial side



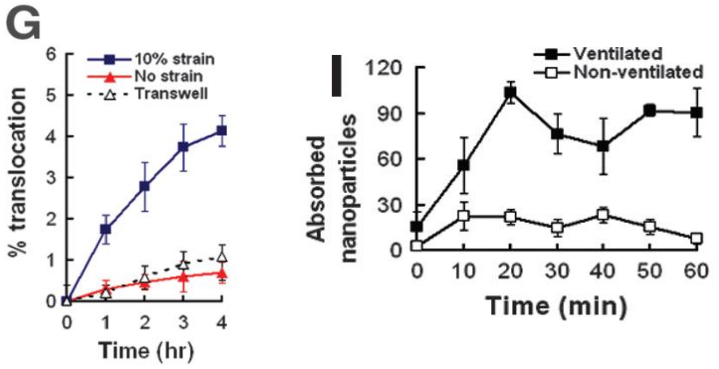
E-Coli introduced to alveolar compartment
Endothelium produces ICAM-1
ICAM-1 recruits neutrophils from the endothelial side
Neutrophils eat e-coli

Uptake of nanoparticles

- Breathing motion greatly enhances the inflammation and reactive oxygen species caused by 12 nm silica nanoparticles.
- Breathing motion also greatly increases of uptake of 20 nm fluorescent particles
- Similar effect observed for a mouse lung kept either static or ventilated
- In vivo experiment also confirms the increased uptake in a breathing real lung. Similar?



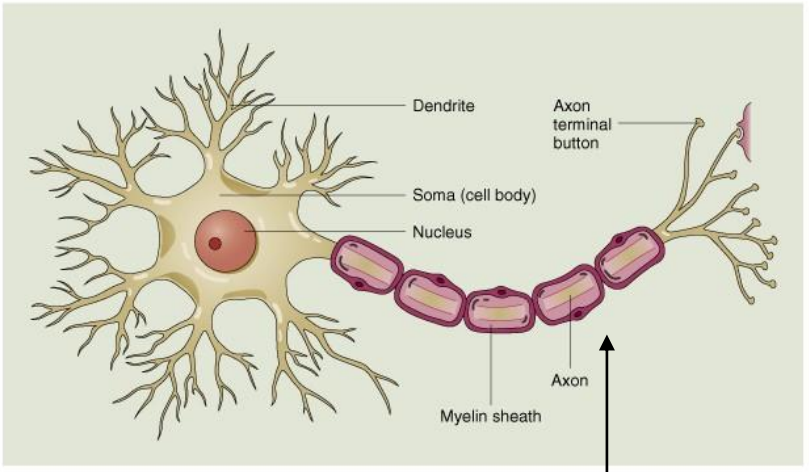
12 nm silica nanoparticles on the chip, breathing vs static



Breathing vs static Intake of 20 nm fluorescent particles on the chip (G) and in a lung (I)

CNS-on-a-chip

- Historical perspective: Squid giant axons (up to 1 mm in diameter) were used in experiments that lead to the discovery of the mechanism of action potentials.
-Macroscopic axons could be interfaced with macroscopic tools.
-Human axons are $\approx$ 1 µm in diameter, suggesting micro/nano sized tools.

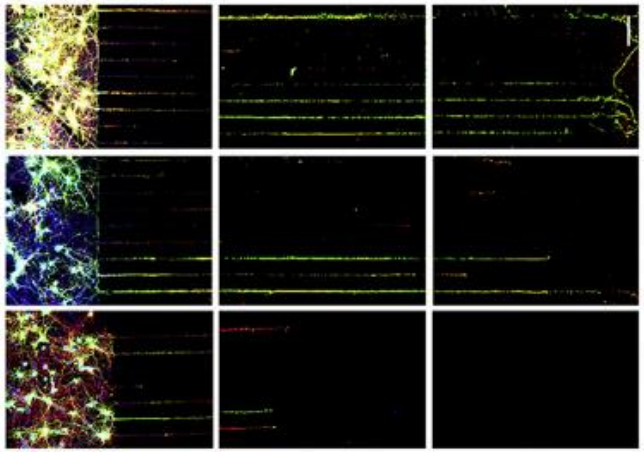


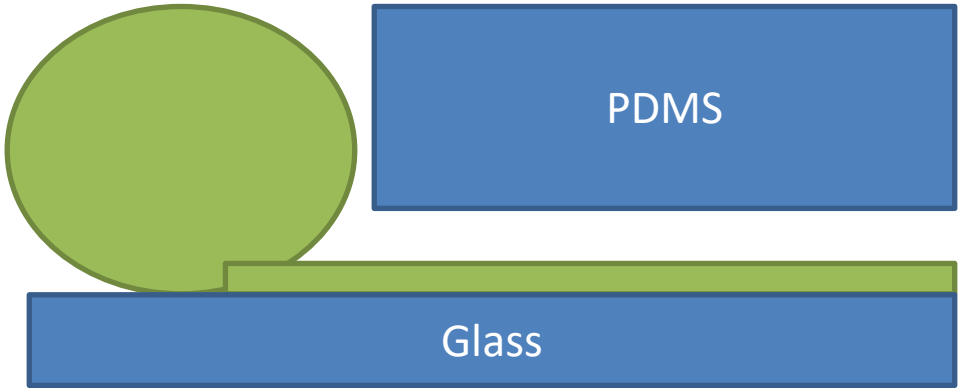
Squid giant axon 1 mm
Human axon 1 µm

CNS: Axon isolation

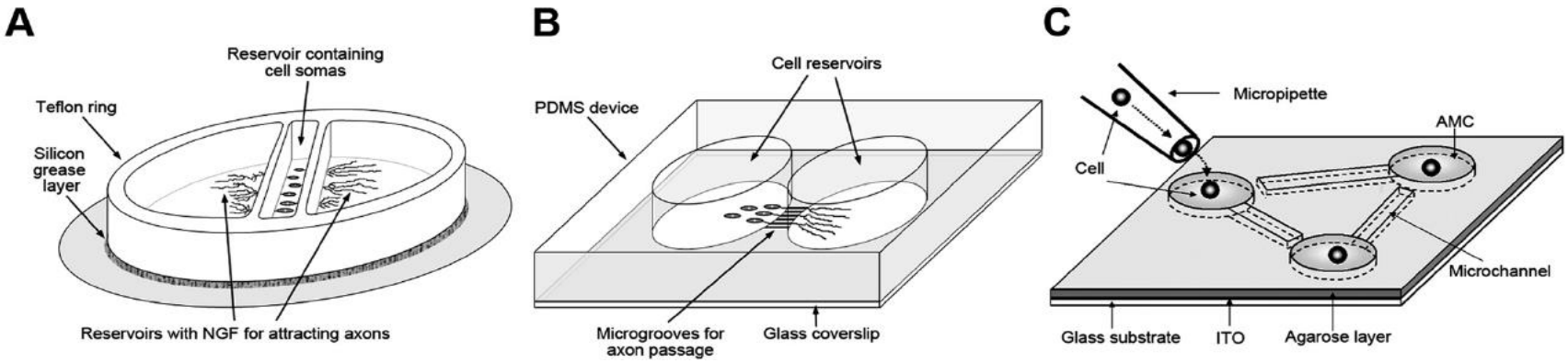
-Size selectivity used to create desired axonal connections to mimic the CNS

Axons (≈ **1µm**) in **3 µm** high microchannels



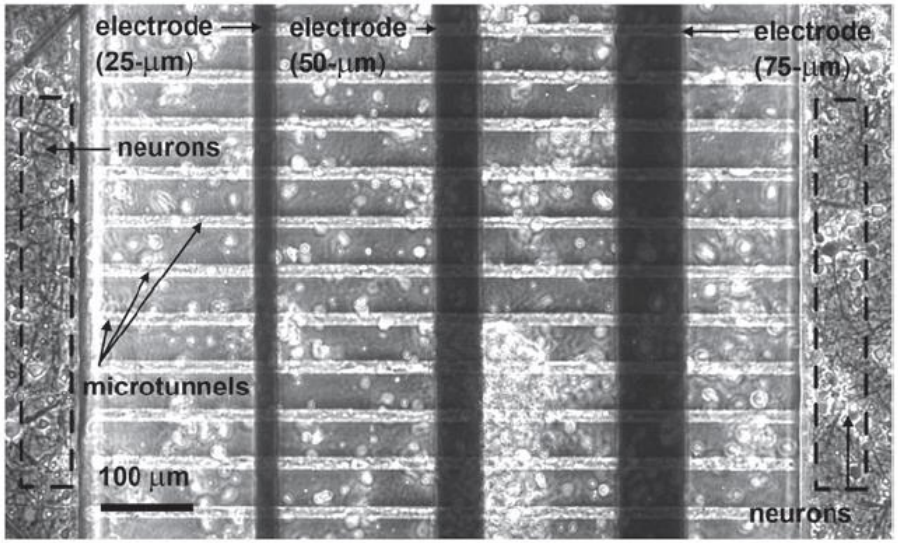


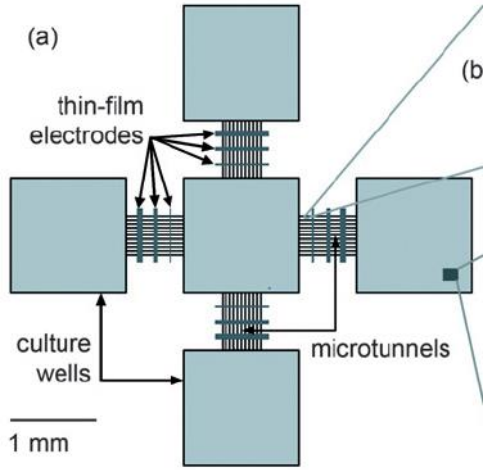
Jokinen et al. J. Neurosci. Methods, 2013

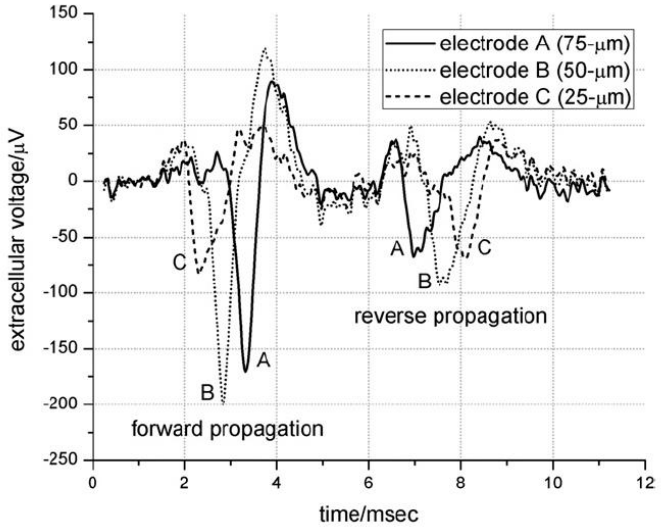


CNS: Integrated sensors

- Microelectrodes can be fabricated to replace external electrodes



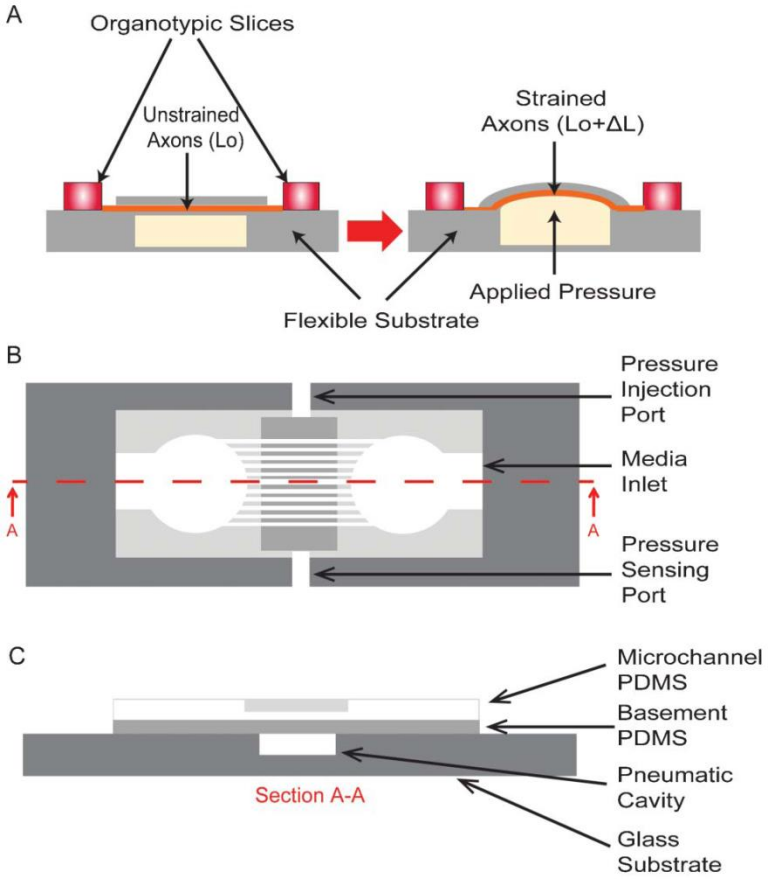




Lab Chip, 2009, **9**, 404–410 | 405

CNS: Disease Model for Axotomy

- Trauma is one of the most studied pathologies on neuro chips.
- Trauma on the axons can be induced by mechanical forces, chemical treatments or heat.

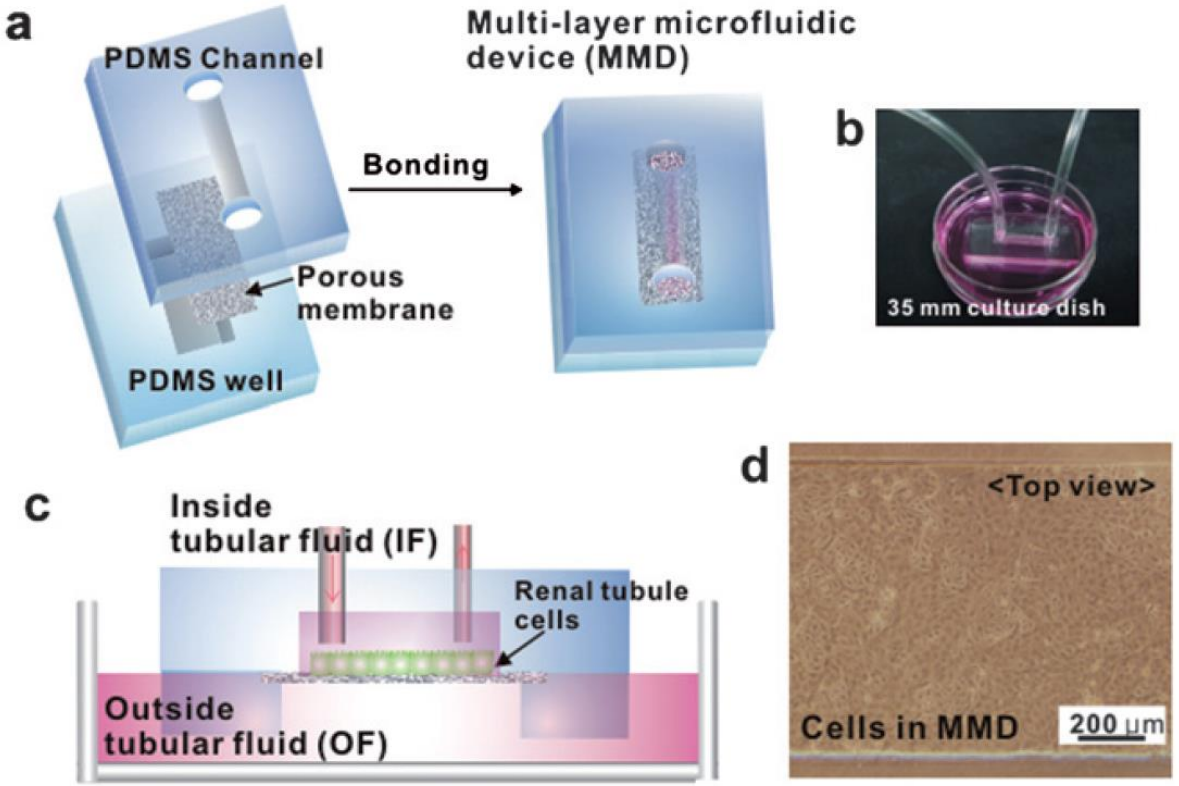


Lab Chip, 2013, **13**, 432–442

Kidney-on-a-chip

Modelled aspects: Architecture of renal tubule. Physiological flow (shear) affects the function greatly.

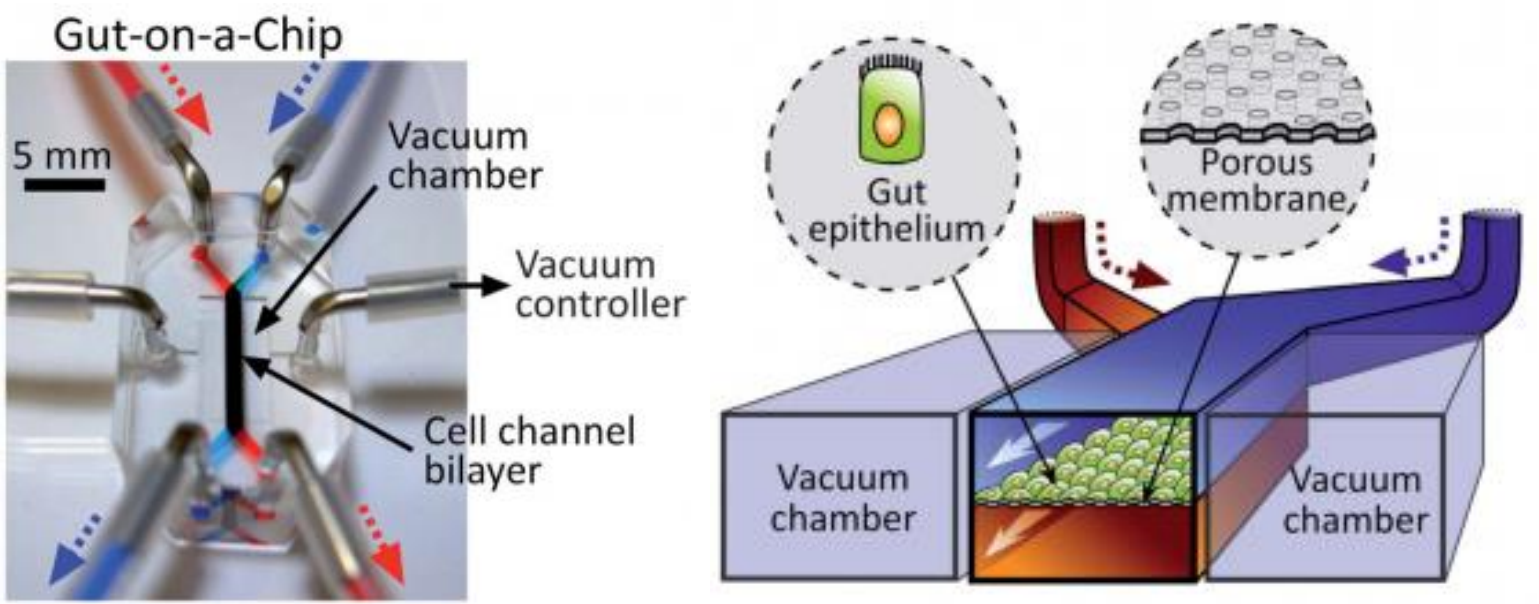
Porous membrane, a monolayer of cells on one side of the membrane



Lab Chip, 2010, **10**, 36–42

Gut-on-a-chip

Modelled aspects: Structure and barrier properties of the digestive tract. Movement and flow effects affect the properties.



Does it look familiar? This is by far the most common organ-on-chip structure, two compartments, separated by a porous membrane, and a confluent layer of one or two types of cells growing on the porous membrane.

Lab Chip 2012; 12 (12):2165-74

A task: come up with a definition for organ-on-chips.

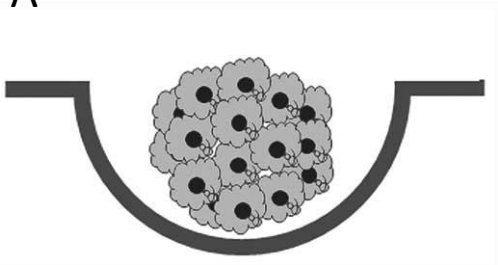
Time for the task about 5 minutes.

Step 1: Write down your own short definition for what counts as organ-on-chip. (You can discuss with others nearby to share thoughts.)

Step 2: Three potential edge cases are shown below. A is a cell spheroid on a non-adherent well plate. B is a transwell insert for well-plates. C. is a brain slice embedded on a microfluidic chip for probing.

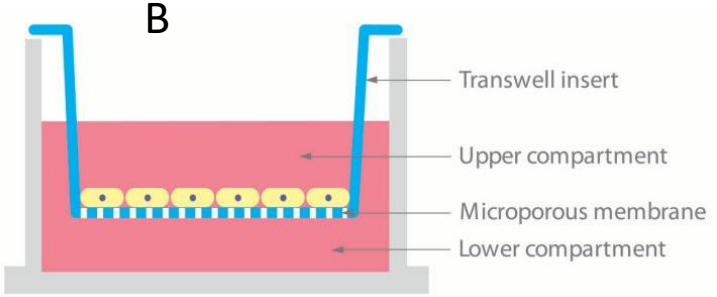
Utilizing the definition you came up with in step 1, how many of these count as organ-on-chips?

A



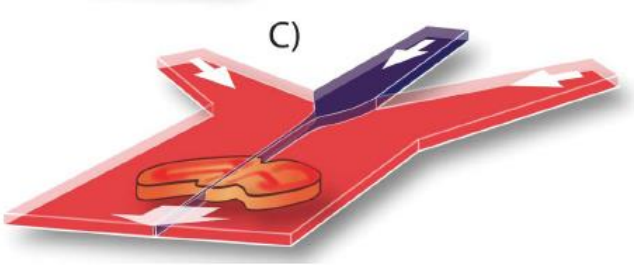
Spheroid

B



Transwell well-plate insert

C)



Brain slice on microfluidic chip

Wikipedia: (accessed 27.5.2018, source is a Scientific American article)
An organ-on-a-chip (OOC) is a multi-channel 3-D microfluidic cell culture chip that simulates the activities, mechanics and physiological response of entire organs and organ systems, a type of artificial organ.

Largely disagree, multi-channel and 3D are unnecessary technical criteria. Entire organs is too high a standard. A type of artificial organ?, only in a very loose sense.

Donald Ingber et al. (DOI: 10.1039/c2lc40089h)
*Organs-on-Chips' in which living **cells** are **cultured** within **microfluidic devices** that have been microengineered to **reconstitute** tissue **arrangements** observed in living organs in order to study physiology in an organ-specific context and to develop specialized in vitro disease models.*

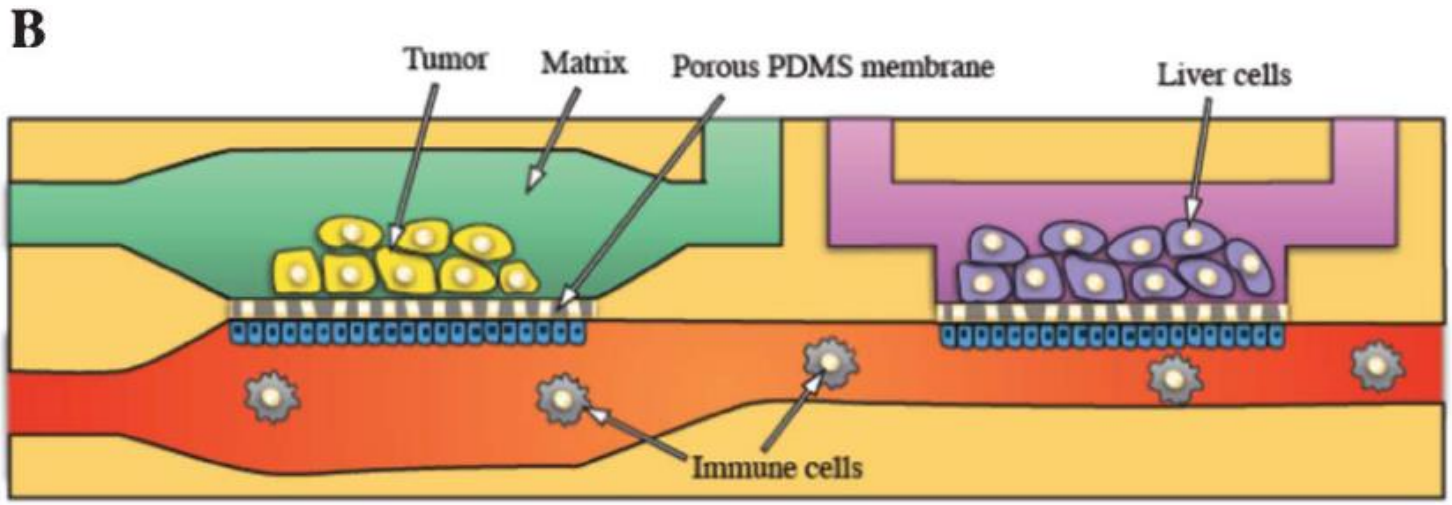
Largely agree with this definition.

Based on either of these, arguably 0/3 were organ-on-chips.
(Spheroid does not (arguably) have cellular arrangement, transwell is not microfluidics, and brain slice does not have cells cultured on chip.)

Having a different definition that includes some (or all) of them would not be unreasonable. The point of the exercise was not to come up with the perfect definition, but to make you think about what is truly core about organ-on-chips and what aspects are incidental.

Basic features of Organs on chip

- One or more different types of cells cultured on a way that mimics some aspect of an organ (or tissue, such as cancer).
- Controlled physicochemical environment, O2 concentration, media composition, etc.
- Possibility for individually addressing different areas of the cell cultures
- Controlled mechanical properties (rigid, soft) and movement (static, “breathing”, flow)
- Surface chemistry: adhesive/nonadhesive
- Controlled interaction between cells: physical contact, soluble factor communication, electrical communication
- Integrated sensors, actuators, stimulating components.



Materials

- Organ-on-a-chip is a hybrid of biological and nonbiological parts.
- Integration is the big challenge: adhesion of cells on surfaces, cytotoxicity of materials, lack of vascularization and other biological support structures, sterilization after the fabrication process…

1. Hard materials (e.g. silicon, glass)
+ Mature fabrication processes
- Fabrication techniques not tuned toward microfluidics
+- Non toxic but unnaturally hard

2. Synthetic polymers (e.g. PDMS)
+ Flexible fabrication for microfluidics
- Softer, but sometimes leach out potentially toxic monomers or additives

3. Biological/biologically inspired materials (e.g. agarose, gelatin)
+ Biological softness, 3D environments
- Limited mechanical strength when standalone
- Can be difficult to fabricate and sterilize

What kind of cells?

- Slices of actual tissue (brain slice, blood vessel)
- Primary cells (taken from a subject)
+ Closest to in vivo conditions
- Require test animal sacrifices

- Immortalized cell lines. For example, HeLa cells (cervical cancer cells taken from Henrietta Lachs in 1951)
- Stem cell lines.
+ Convenient
+ Do not require test animals
- Cell line deviations and contaminations
- Probably less accurate models for in vivo processes

- Patient derived stem cell lines, induced pluripotent cells
+ Patient specificity (“Patient-on-a-chip”)
- Difficult biology
- Still in early development stages

Some points about fabrication

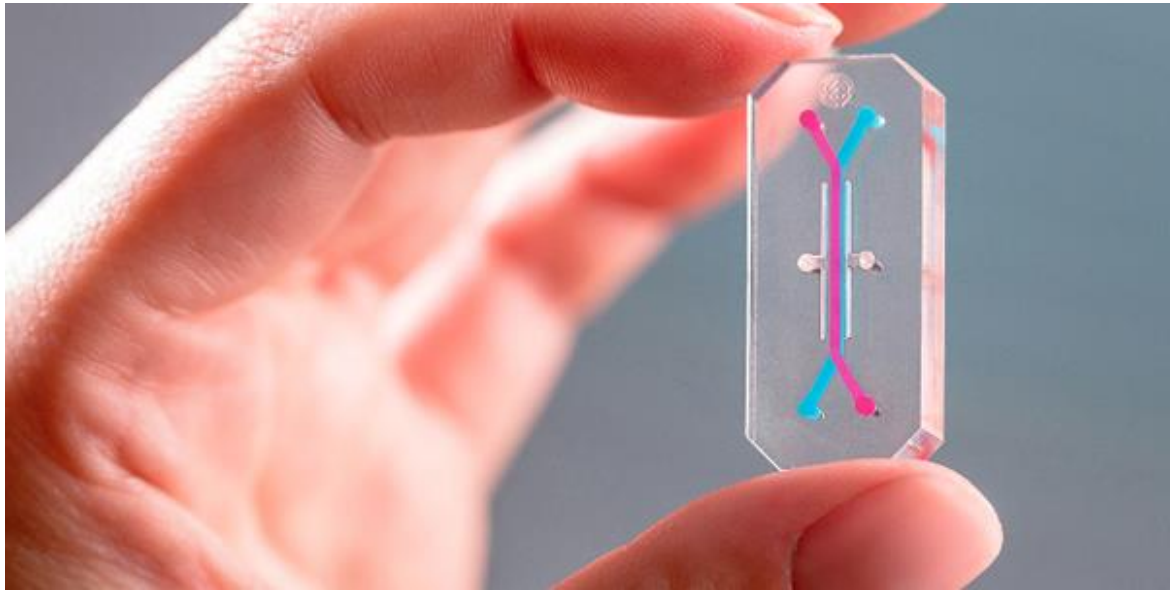
- The examples in this lecture were made by traditional microfabrication techniques. They are 2.5D, layered 2D designs.
- 3D printing is another method. It can print both the cells and the non-living parts.

- Unlike normal fabrication, the cells are living “intelligent” building blocks that will self-organize to the correct structure if they are initially organized in a roughly correct environment.

- One problem is that the materials field is divided into many categories: 1. Prototyping materials (PDMS) 2. Mass production materials (PMMA, COC) 3. Materials familiar to biomedical field (glass, polystyrene) 4. Actual biological material of tissues and other structures.

Organ-on-chip commercialization

- Harvard startup Emulate raised 28 million of venture capital for making a product out of Lung-on-Chip and other organ on chips. They now have products for many organs and collaborations with multiple big pharma companies
- Many other companies selling chips and kits for Organ-on-chips.
- Many, or most, pharma companies are testing them for drug development, but they are not (yet) a part of the main development process.



https://emulatebio.com

Review

- Organs-on-chips are microfluidic models of an aspect of organ or tissue
- A scaffold (or chip) of non-living matter helps to organize living matter into an arrangement that is the same or close to the arrangement those cells would take in vivo.
- An intermediary between well plates and animal models.
- Human or animal cells

- Still a developing field, but there are high hopes that Organ-on-chips would provide more accurate predictions about in-vivo drug effects than traditional in-vitro methods.