



UNIVERSITÀ
DEGLI STUDI
DI TERAMO

Corso di Laurea Magistrale in Biotecnologie Avanzate
Corso di Laurea Magistrale in Reproductive Biotechnologies
AA 2023-2024

Microfluidic Devices: Organ-On-a-Chip



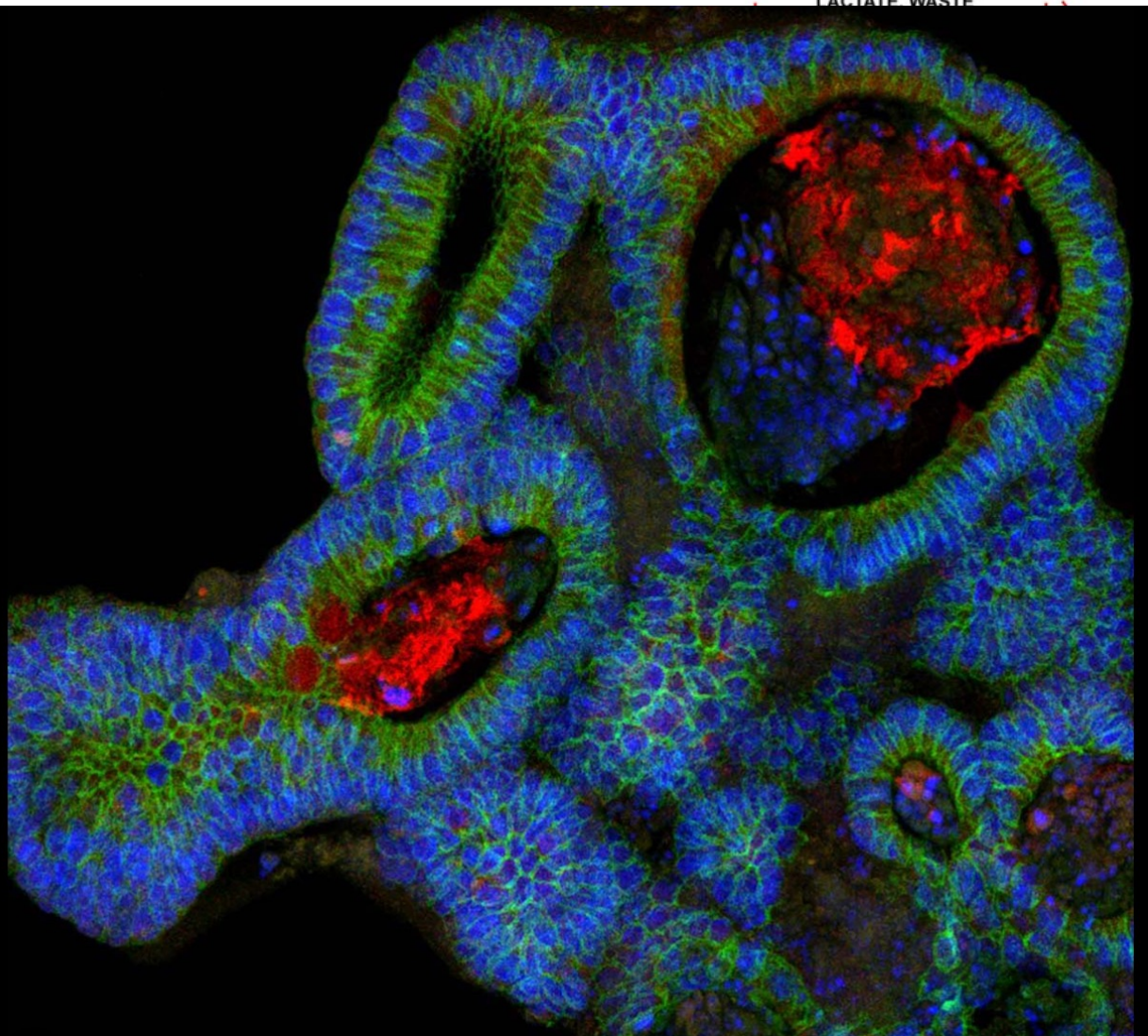
Creating environments for in vitro cell growth

What are the disadvantages of 2D vs 3D cell culture?

	Disadvantages of 2D cell culture	Disadvantages of 3D cell culture	
High side	Cells forced into planar shape, does not mimic native structures	Slower culture formation due to physical restraints of the matrix (hours to days)	ess vo
	No cellular microenvironment	More complex procedures	
L access n	Lacking complex cell-cell and cell-environment interactions	Higher reagent cost	medium ts ivo
	Unrestricted access to essential compounds, unlike <i>in vivo</i>	Fewer commercially available tests	
	Different cell morphology and molecular mechanisms compared to <i>in vivo</i>		
	Stiffness of surrounding tissue not replicated		

D**METABOLIC GRADIENT**

LACTATE WASTE



INNER REGION

OXYGEN GRADIENT

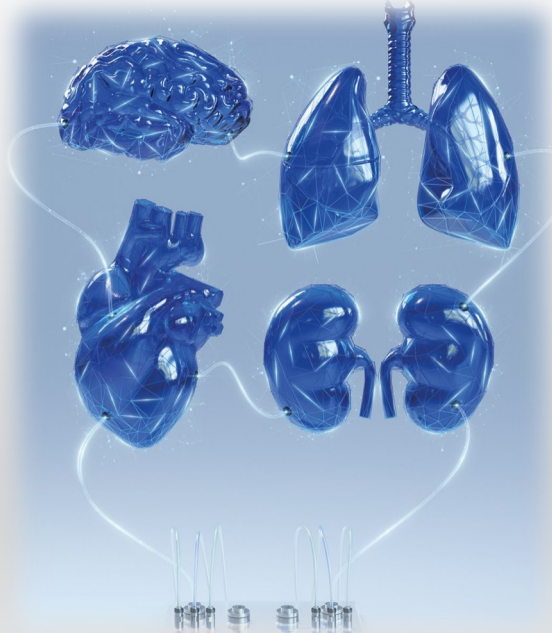
Differences	Spheroid	Organoid
Cell types	Multiple cell types including cell lines, tumor cells, primary cells and mixtures of cells	At least one endothelial and one mesenchymal cell type, including stem cells, induced pluripotent cells and tumor cells
Architecture	Resemblance to single tissue or 3D cellular architecture	Resemblance to multiple tissues or an organ
Form	Layers of heterogenous proliferating, necrotic or quiescent cells	Complex structures of differentiating cells
Assembly	Self-assembly with cell adhesion and cell-to-cell aggregation	Self-assembly of differentiating cells in response to physical and chemical cues
Organization	Self-organization in certain models	Self-organization into complex structures and patterning
Supplements	With or without extracellular matrix and growth factors, does not require expensive scaffolds	Requires growth factors, extracellular matrix with or without expensive scaffolds; organoids without scaffolds require additional accessories
Time and complexity	Lower complexity, less time to generate, less expensive than organoids	Higher complexity, longer to generate, more expensive than spheroids



ORGAN-ON-A-CHIP

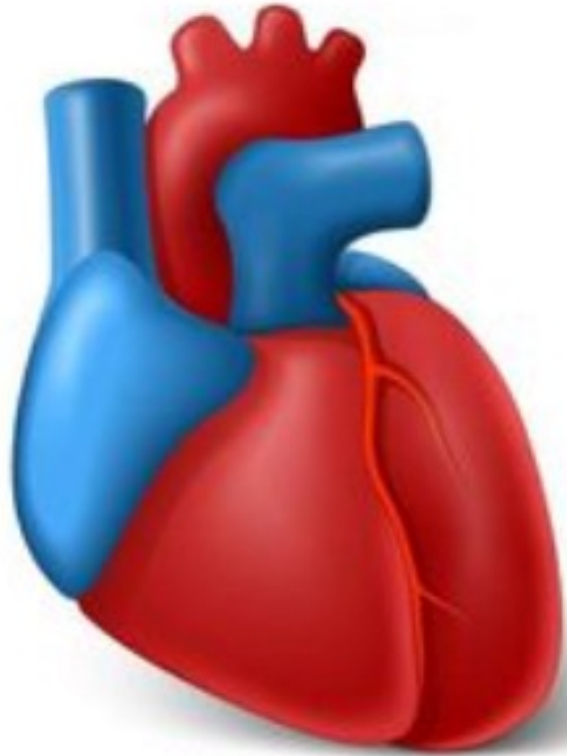


MULTI-ORGAN-ON-A-CHIP



BODY-ON-A-CHIP





What is an Organ-on-a-chip (OoC)?

- **SYNONIMS:** Also known as 'tissue chips' or microphysiological systems
- **DEFINITION:** microdevices engineered to contain cells and tissues and to model or mimic organ structures, functions and reactions to biological conditions, stressors or compounds



OoC: Where needs come from?

Animal models have contributed to:

- understanding of the physiology and disease
- development of new medicines

Frequent discordance between animal and human studies have been recorded

MUST HAVE!

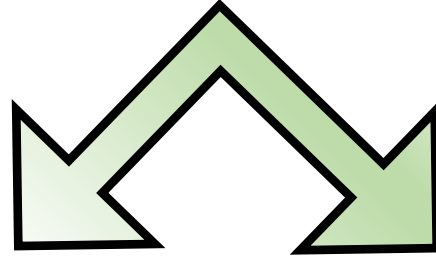
modelling and testing platforms more predictive of human responses

Indeed, drug candidates may be terminated for lack of efficacy in animals, or discovery of hazards or toxicity in animals that might not be relevant to humans

Let's give some numbers...

more than 80% of investigational drugs fail in clinical testing:
60% of those failures due to lack of efficacy
30% due to toxicity

Key features for setting up OoC



TECHNICAL

BIOLOGICAL



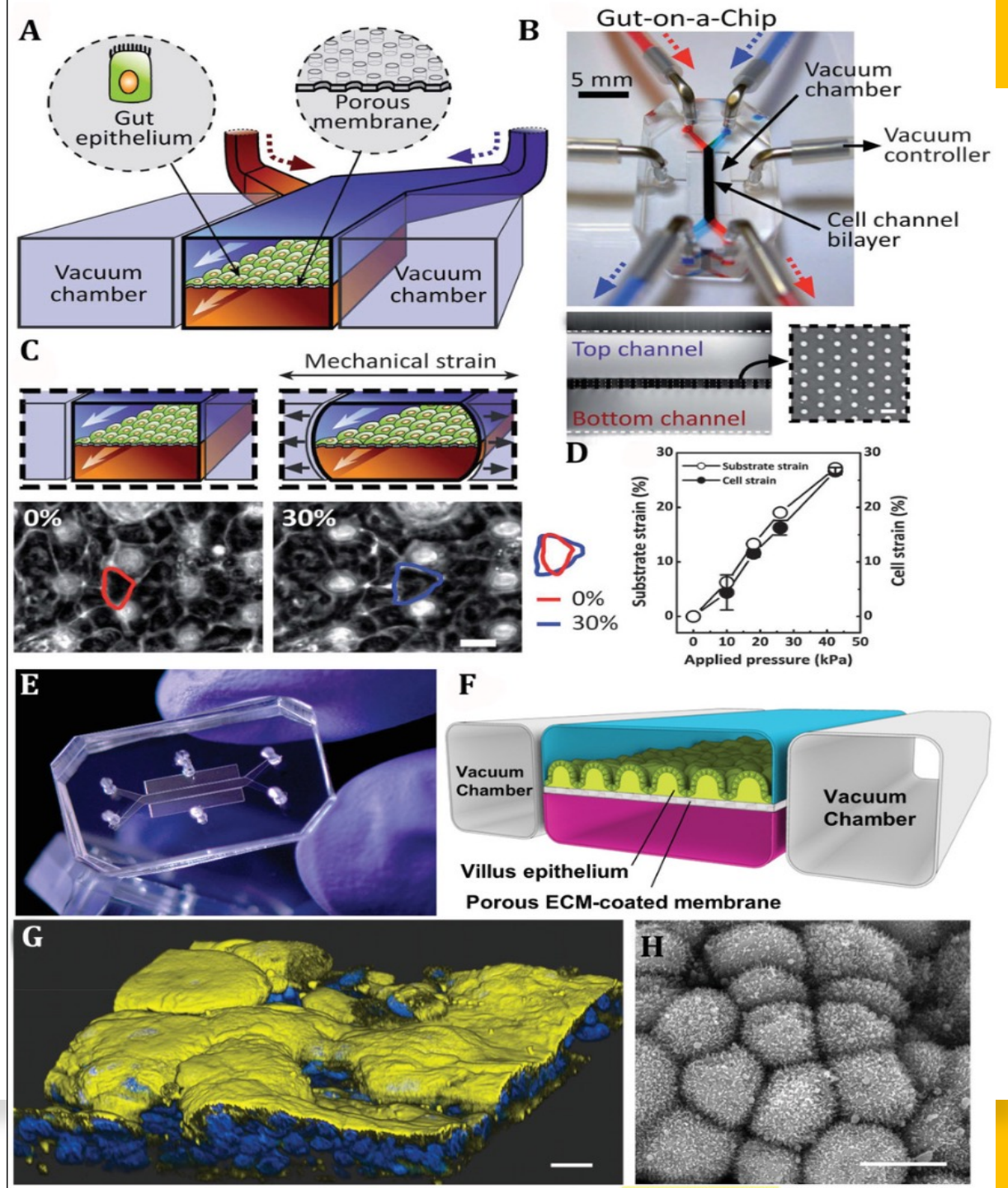
- Platform design
- Platform fabrication

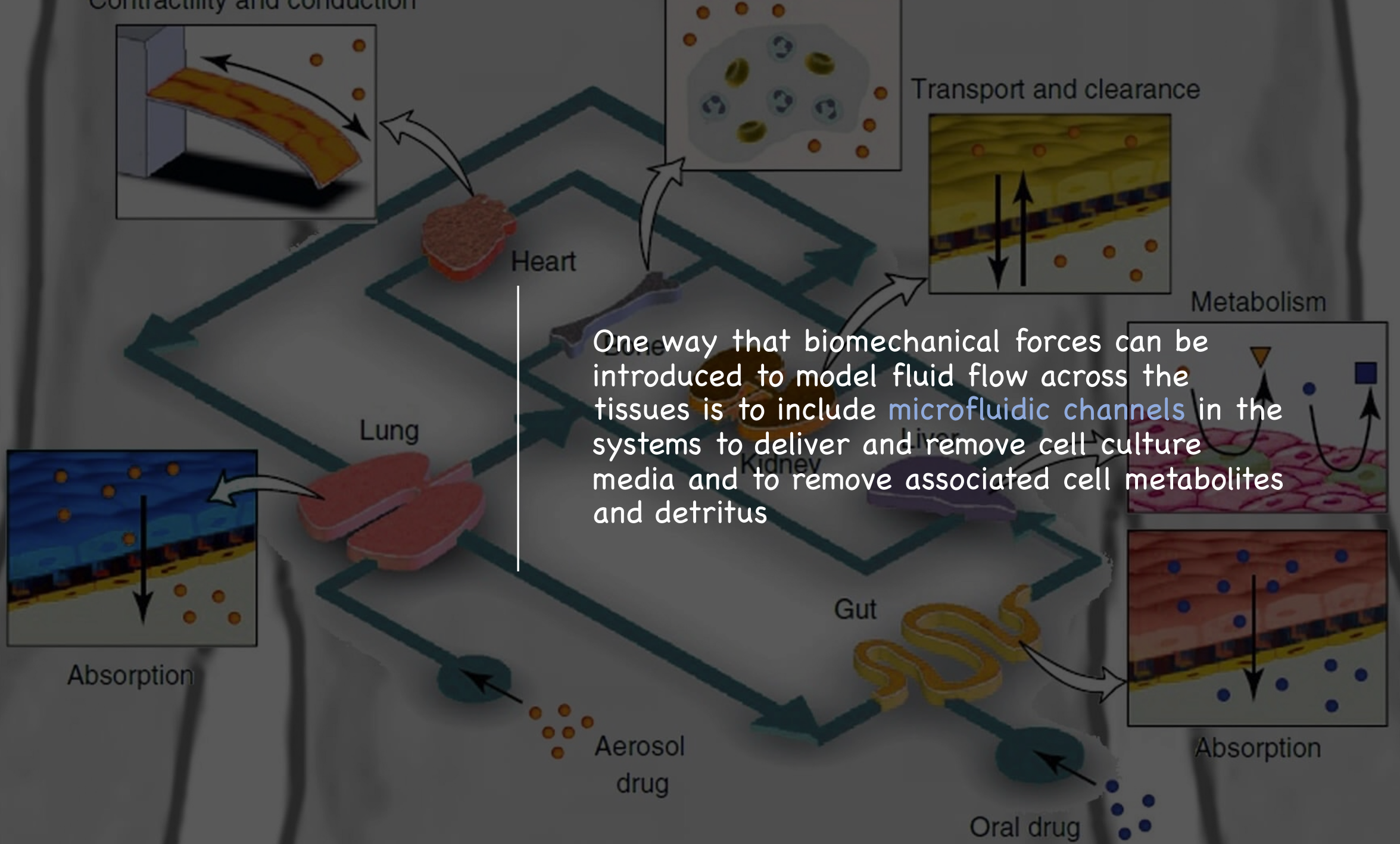
Conceptual OoC design

- OoCs range from devices the size of a USB thumb drive to larger systems that reflect multiple linked organs within the footprint of a standard 96-well laboratory plate.

FEATURES:

- the 3D nature and arrangements of the tissues on the platforms
- the presence and integration of multiple cell types to reflect a more physiological balance of cells (parenchimal, stromal, vascular and immune cells)
- the presence of biomechanical forces relevant to the tissue being modelled





One way that biomechanical forces can be introduced to model fluid flow across the tissues is to include **microfluidic channels** in the systems to deliver and remove cell culture media and to remove associated cell metabolites and detritus

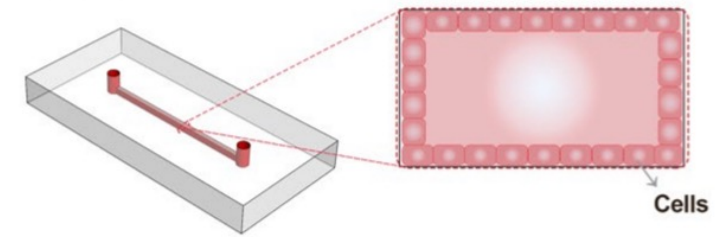
OoC: Geometry and Dimensions

Classified based on numbers and organization of channels/compartments

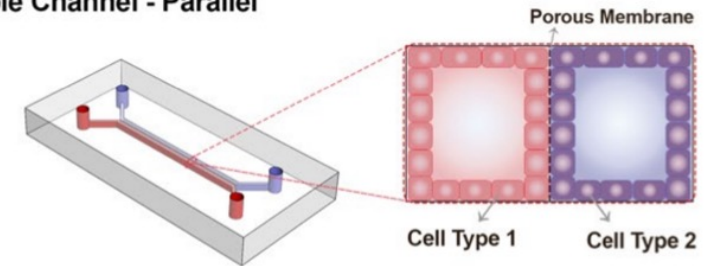
Double channel design is mostly used where one compartment was used to mimic the blood vessels and the other compartment(s) for the actual tissue cells

Porous membranes are usually polymeric

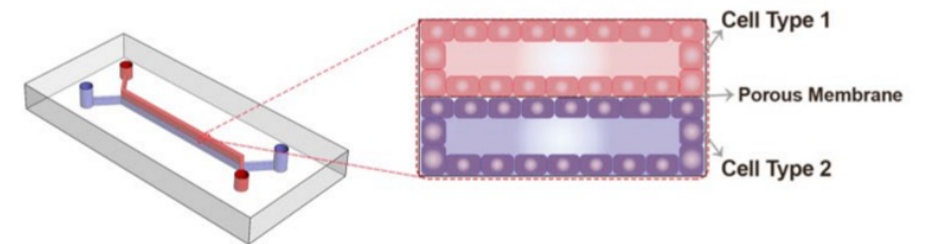
Single Channel



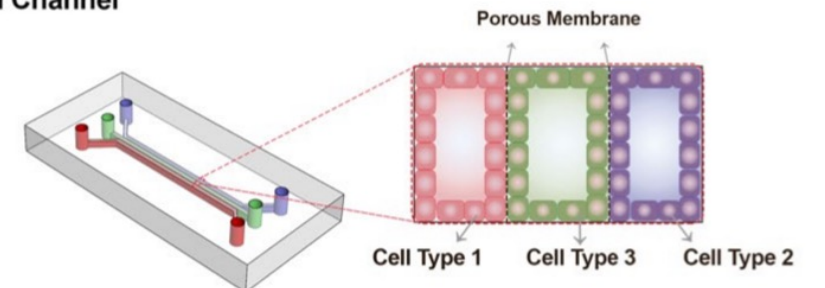
Double Channel - Parallel



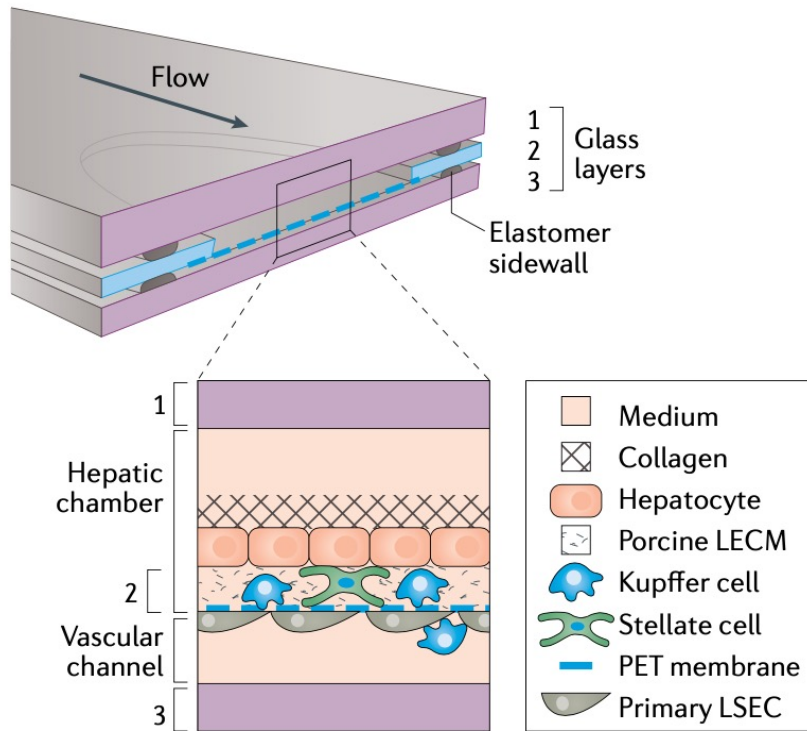
Double Channel - Sandwich



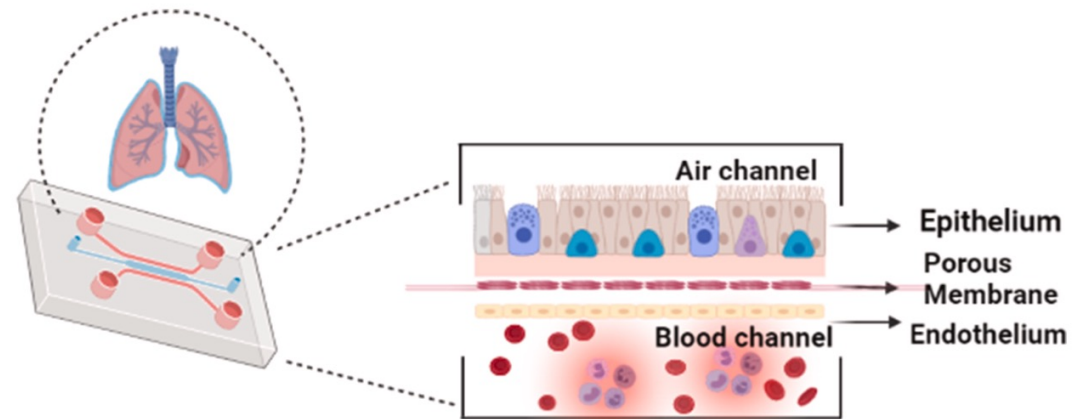
Multi Channel



Example: OoC architecture



Low et al., 2020



Singh et al., 2022

OoC: Channels and ports

- The shapes and diameters of channels vary extensively:
circular and rectangular types
From 10mm to 20um
- Ports for inflow and outflow design must keep sterility
circular and rectangular types
From 10mm to 20um
- Bubble traps must be incorporated

Ex. A lung-on-a-chip with vacuum channels running alongside a porous membrane onto which lung alveolar cells were seeded on one side and lung endothelial cells were seeded on the other. Rhythmic application of the vacuum caused stretching and relaxation of the cell-lined membrane and mimicked the biomechanical forces associated with breathing. Adapeted also for gut, heart, blood-brain barrier and kidney glomerulus.

OoC: Clogging mechanisms

Clogging is defined as the interruption of flow due to the aggregation of particles

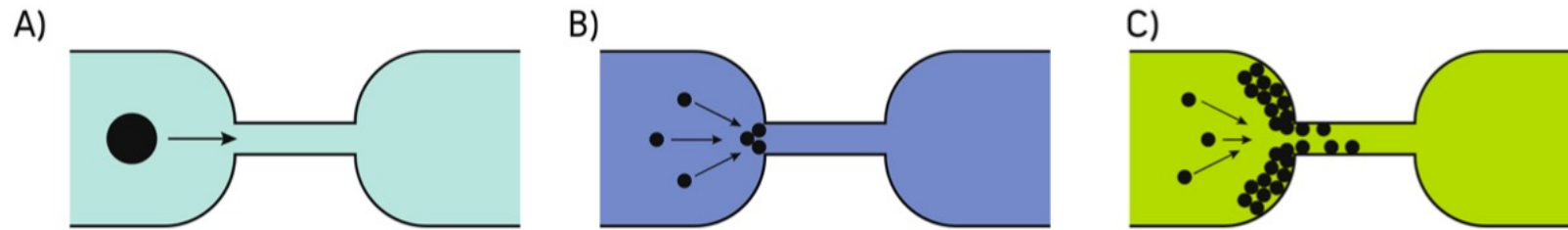


Figure 4. Clogging mechanisms: (A) sieving, (B) bridging, and (C) aggregation.

Tajeddin et al., 2021

SIEVING: Particles are larger than dimension of channels

BRIDGING: Particles are smaller than the channel and form an arch-shape along the width of the channel due to the steric effects

AGGREGATION: The aggregated layer grows as a result of competition between hydrodynamic, diffusive, and colloidal effects

OoC: Fabrication Materials

Polydimethylsiloxane:
a silicon-based elastomer

PDMS



- ✓ Transparency
- ✓ Biocompatibility
- ✓ Low cost
- ✓ Flexibility
- ✗ Hydrophobic
- ✗ Slightly Fluorescent

soda lime, Quartz, Borosilicate
They are a mixture of silicon dioxide (SiO₂), the base material of glass, with other oxides, such as CaO and MgO

Glass



- ✓ Transparency
- ✓ Biocompatibility
- ✓ hydrophilicity
- ✗ Gas impermeability
- ✗ Inflexible

polymethyl methacrylate (PMMA)
or copolymers (COC)

Thermoplastic



- ✓ Biocompatibility
- ✓ Easy Fabrication,
- ✓ Low cost
- ✗ Poor gas permeability
- ✗ Slightly Fluorescence
- ✗ Inflexible

OTHER MATERIALS: Hydrogels, silicon, metals (titanium, gold)

PDMS



- ✓ Transparency
- ✓ Biocompatibility
- ✓ Low cost
- ✓ Flexibility
- ✗ Hydrophobic
- ✗ Slightly Fluorescent

PDMS is the **most common material** used for the fabrication of microfluidic devices, and OOCs in particular. It is a silicon-based elastomer and has extremely advantageous properties, namely **economic feasibility, transparency, flexibility, oxygen permeability, and biocompatibility**. It also shows good compliance with various microfabrication techniques, such as soft lithography or molding.

On the other hand, there are **some properties that limit the use of PDMS** and motivate the search for alternatives. The **absorption of hydrophobic molecules** is a drawback that negatively affects the results of toxicity, efficacy, and also PK/PD (pharmacokinetics/pharmacodynamics) predictions. It is also **fluorescent** to some degree and unsuitable for working with organic solvents.

There are increasing attempts to improve the properties of PDMS-made chips by surface modifications using plasma treatment, UV treatment, and coating. There are various coatings that can reduce the surface energy of PDMS; those include some metals/metal oxides.

Glass



- ✓ Transparency
- ✓ Biocompatibility
- ✓ hydrophilicity
- ✗ Gas impermeability
- ✗ Inflexible

One of the **oldest materials** in the development of microfluidic devices is glass. In general, there are three types of glass used in this field: (i) **soda lime**, (ii) **quartz**, and (iii) **borosilicate**. They are a mixture of silicon dioxide (SiO_2), the base material of glass, with other oxides, such as CaO and MgO .

Many advantages have been reported on the use of glass in microfabrication, and OOCs in particular, such as **transparency**, **resistance to mechanical stress**, **hydrophilicity**, and **biocompatibility**. In addition, glass has been reported to have **lower drug absorptivity** compared to PDMS.

On the other hand, one **major problem** that can lead to channel plugging is the **low gas permeability of glass**. Therefore, special care must be taken in the design and fabrication, e.g., by the use of **bubble traps/removers**. Glass has **high cost** of fabrication and is **time-consuming**.

However, there are certain topics for which the use of glass microfluidics is highly recommended, such as the prediction of **PK and PD for drug testing** and can be **advantageous in anaerobic studies**.

Thermoplastic



- ✓ Biocompatibility
- ✓ Easy Fabrication,
- ✓ Low cost
- ✗ Poor gas permeability
- ✗ Slightly Fluorescence
- ✗ Inflexible

Recently, thermoplastics have been increasingly proposed for the fabrication of microfluidic devices due to the limitations of PDMS and glass-based chips

There are interesting properties that make thermoplastic polymers attractive for OOCs, including **low cost**, **low density**, **biocompatibility**, and **easy fabrication**. As they have linear and branched molecules, they are **more resistant to pressure and temperature fluctuations**, which also makes them chemically stable and suitable for biomedical/biochemical studies. The most used are polymethylmethacrylate (PMMA) or copolymers (COC).

On the other hand, there are **some limitations** in the use of thermoplastic polymers: (i) **not all** manufactured polymers **are transparent**, which makes microscopic observation or imaging impossible; (ii) **some have strong autofluorescence** properties and are not suitable for detection purposes; (iii) they **have poor gas permeability**, which has a negative impact on long-term cell culture (such as OOCs).

OoC: Fabrication Methods

BOTTOM-UP

the microstructures need to be considered and the cells are seeded into a microenvironment (usually hydrogels) to develop their vascular networks

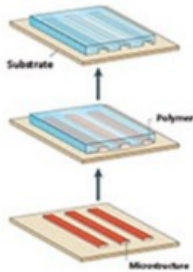
TOP-DOWN

the microstructure (microvessels) is created and then the cells are seeded.

BOTH

Sometimes a hybrid approach is taken that includes both the bottom-up and the top-down approaches

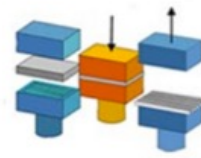
Elastomers



Soft-lithography

- Combination of photolithography and molding
- Suitable for elastomeric materials

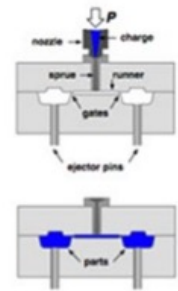
Thermoplastic materials



Hot Embossing

- Requires master mold fabrication
- Suitable for polymeric materials

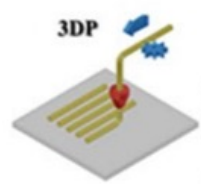
Polymers



Injection Molding

- Requires master mold fabrication
- Low-cost high precision microfabrication suitable for batch production

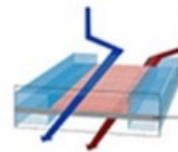
Elastomers



3D Printing

- Supports both additive and subtractive manufacturing
- Used for master preparation

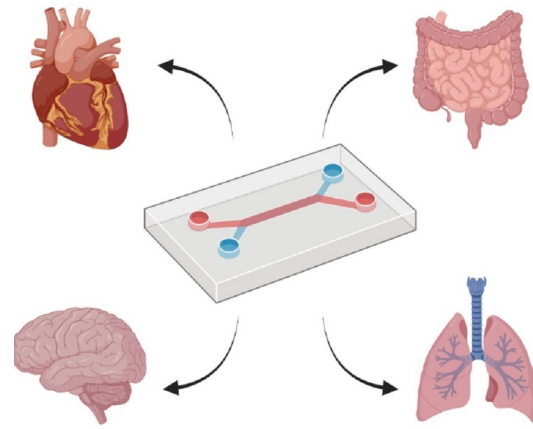
Creative Methods



- Easy implementation methods without high cost facilities
- Suitable for preliminary experiments

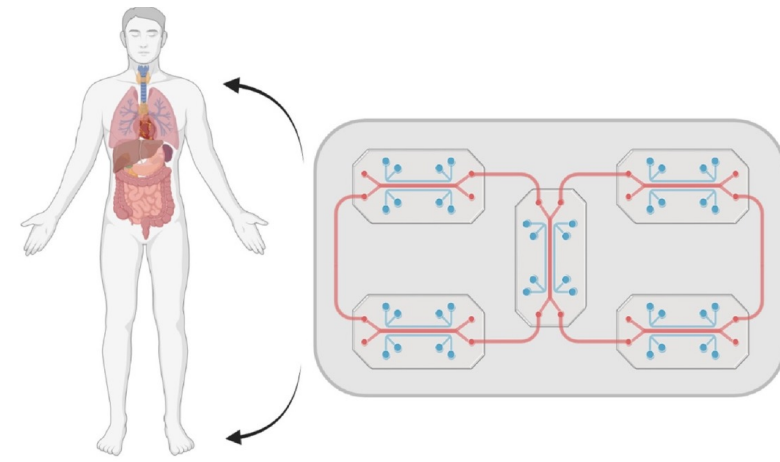
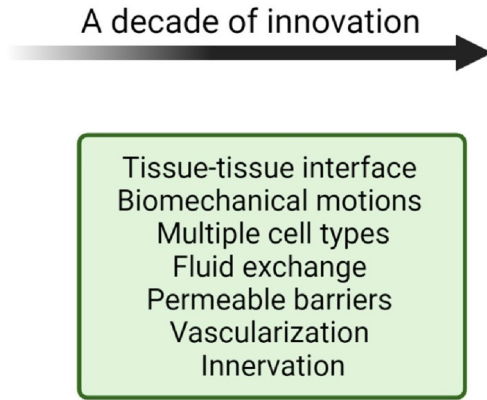
Method		Cost	Facility Requirement	Precision	Capability for Surface Treatment
3D printing	Additive, Removal, and Patterning	Yellow	Red	Yellow	Green
Soft lithography	One step patterning and removal and molding	Yellow	Yellow	Green	Green
Hot Embossing	One step patterning and removal and molding	Yellow	Red	Green	Yellow
Injection molding	One step patterning and removal and molding	Green	Red	Green	Red
Miscellaneous	—	Green	Green	Red	Green

■ Positive; ■ Moderate; ■ Negative.



Single organ chips

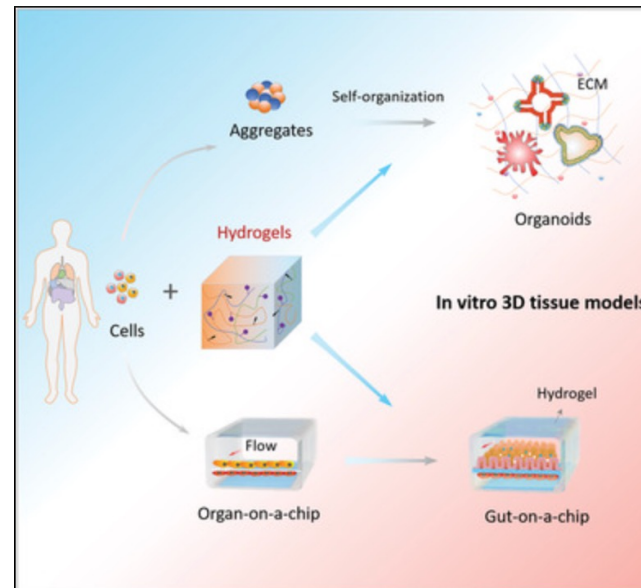
Organ-level physiology and functionality
Disease modeling
Toxicological assessment



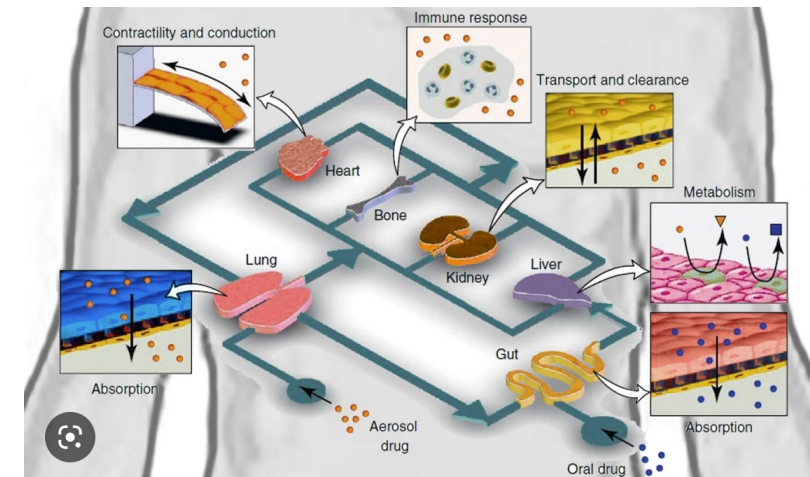
Interconnected organ chips

PK/PD modeling
Systemic modeling of diseases
Drug screening
Personalized medicine

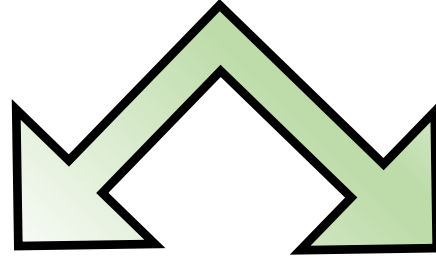
Hydrogels are another new material in the field of OoCs. They are mainly used as bio-scaffolds for cell culturing which is close to the extracellular matrix (ECM)



Trends in Biotechnology



OoC: Key features for setting up OoC



TECHNICAL

BIOLOGICAL



Cell source
Cell scaffolds

Understanding tissue composition and scaffold or ECM influence cellular functions and architecture

Cell source

PRIMARY CELLS: The clear advantage of using cells from human donors is that the cells capture the phenotype of the mature adult state

iPS CELLS: solution to cell sourcing difficulties for tissue chips.
Allow for creation of isogenic cell lines for genetic disorders

Cell scaffolds

It is important to reconstitute a physiological environment, conducive to cell growth.
The choice of the decellular scaffolds or hydrogels (natural or synthetic) should be tested as a function of the tissue type

OoC applications

TOXICITY

Assessing response
to therapeutics with known
or unknown mechanisms
of action

MODELLING DISEASES

Modelling organs
and tissues from
individual donors
(healthy and diseased)

MODELLING CELL RESPONSE TO STIMULI

Investigating the responses of
these tissues to environmental
perturbations

OoC: Toxicity assessment

Current methods:

1. High-throughput cell culture assays

Limitation:

the method cannot replicate a complex systemic response to a compound

2. Animal models which can model complex responses

Limitation:

- the method may not provide an accurate prediction of effects in humans as anatomic and physiological aspects may hugely differ among different animals. Only for prediction studies about absorption, distribution, metabolism and excretion (ADME) of chemical substances
- The method is not applicable to predict toxicity of large molecules (mw 900Da) characterizing new interesting active biological compounds

Difficulty Translating findings from animals to humans can be seen in high-profile phase I clinical trials

The use of OoC might allow to overcome some of the above limitations

Example of toxicity assessment with OoC

For the heart, which is another important target organ of toxicity, a number of **heart-on-a-chip systems** have been developed that model the complex matrices of cardiomyocytes, (cardiac) fibroblasts, endothelial cells and vasculature that interact in vivo in a highly ordered manner, which can be easily perturbed by drugs, drug-drug interactions or off-target side effects.

Heart-on-a-chip, specifically, cardiac valves, have been bioengineered to assess the off-target cardiac side effects of drugs that influence dopamine/ serotonin production/reuptake (pergolide). Pergolide is used in clinical treatment for psychiatric disorders such as Parkinson disease

https://www.youtube.com/watch?v=CpkXmtJOH84&ab_channel=TED

OoC: Disease modelling in vitro

With iPS

Advantages: High plasticity and differentiation potential rendering broad disease modelling application

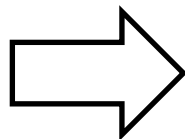
Limitations: The difficulty to produce and adequate number of mature, differentiated cells with the necessary purity of many tissues.

With tumor cells

Advantages: accurate modelling

Limitation: low plasticity. Need of the specific cellular model for the targeted tumor

A STEP FORWARD

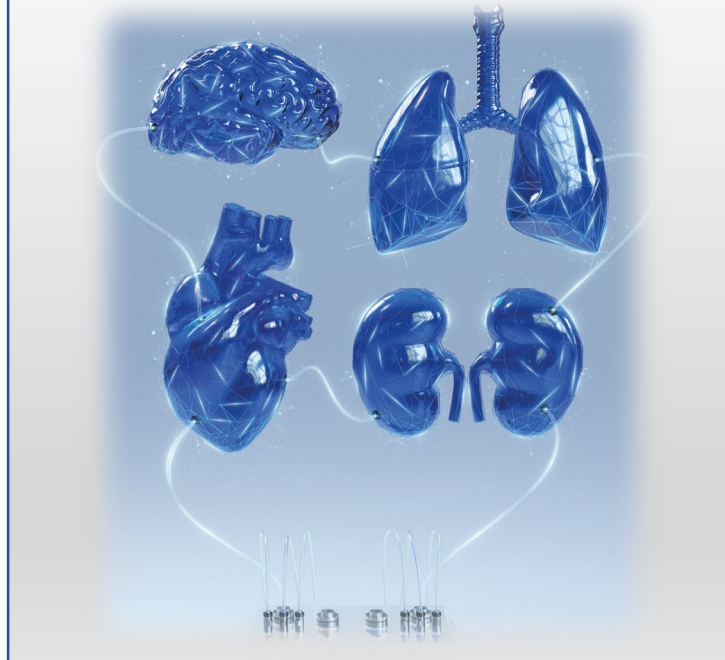


PATIENT-ON-A-CHIP or YOU-ON-A-CHIP

TARGETED Disease modeling (and also therapy) with chip devices bearing patient-derived primary or iPS cell derivatives

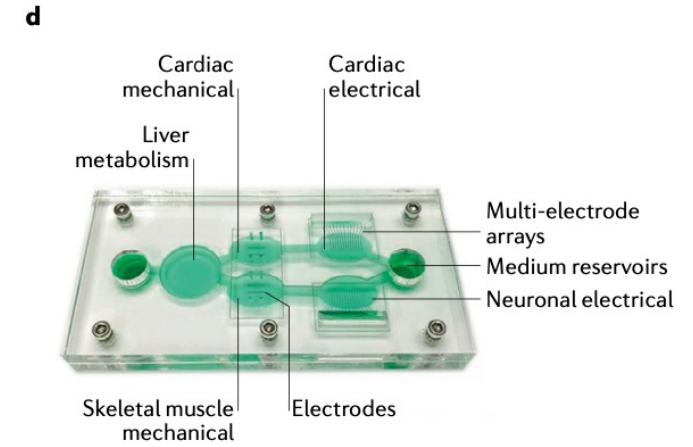
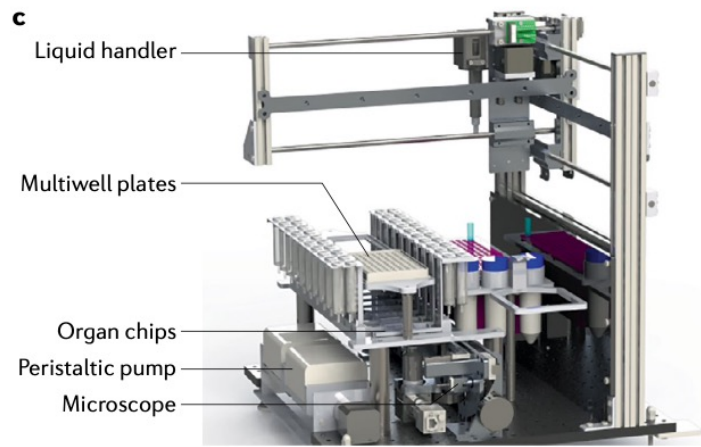
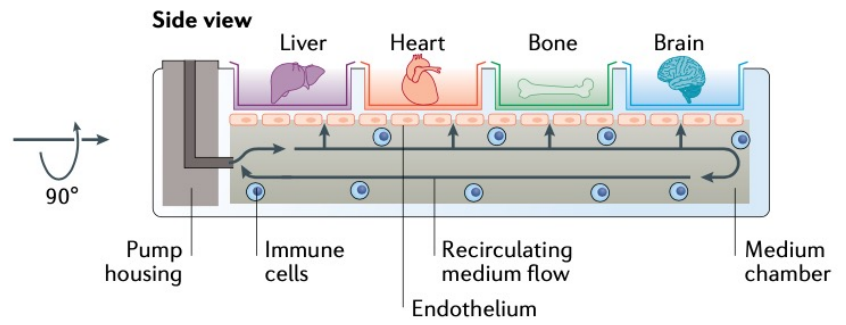
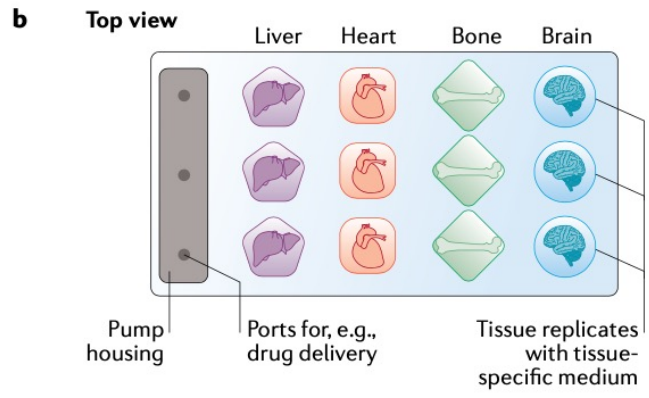
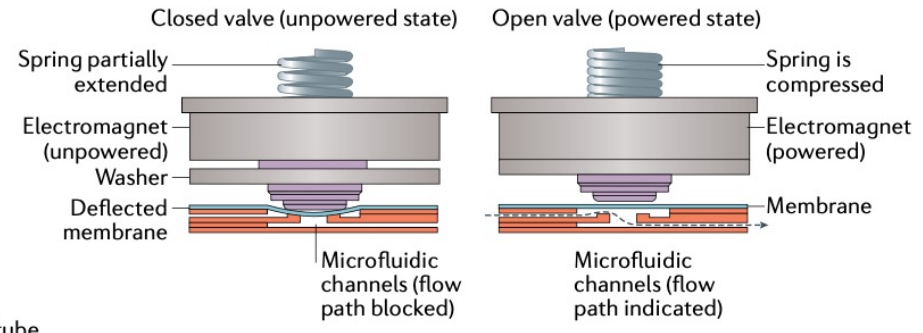
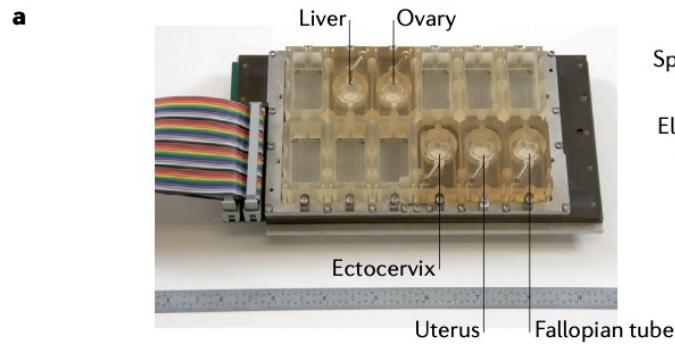


MULTI-ORGAN-ON-A-CHIP



mOoC:

Systems linking multi-organ systems



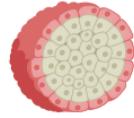
Example: a generic 2-organ system

a Conceptualization and design

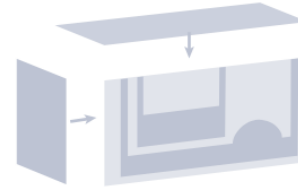
Organ 1: barrier type.
Cells cultured on ECM-coated membrane



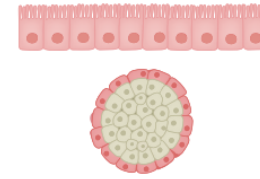
Organ 2: 3D-cultured tissue, e.g. organoid, with parenchyme



b Material selection and fabrication

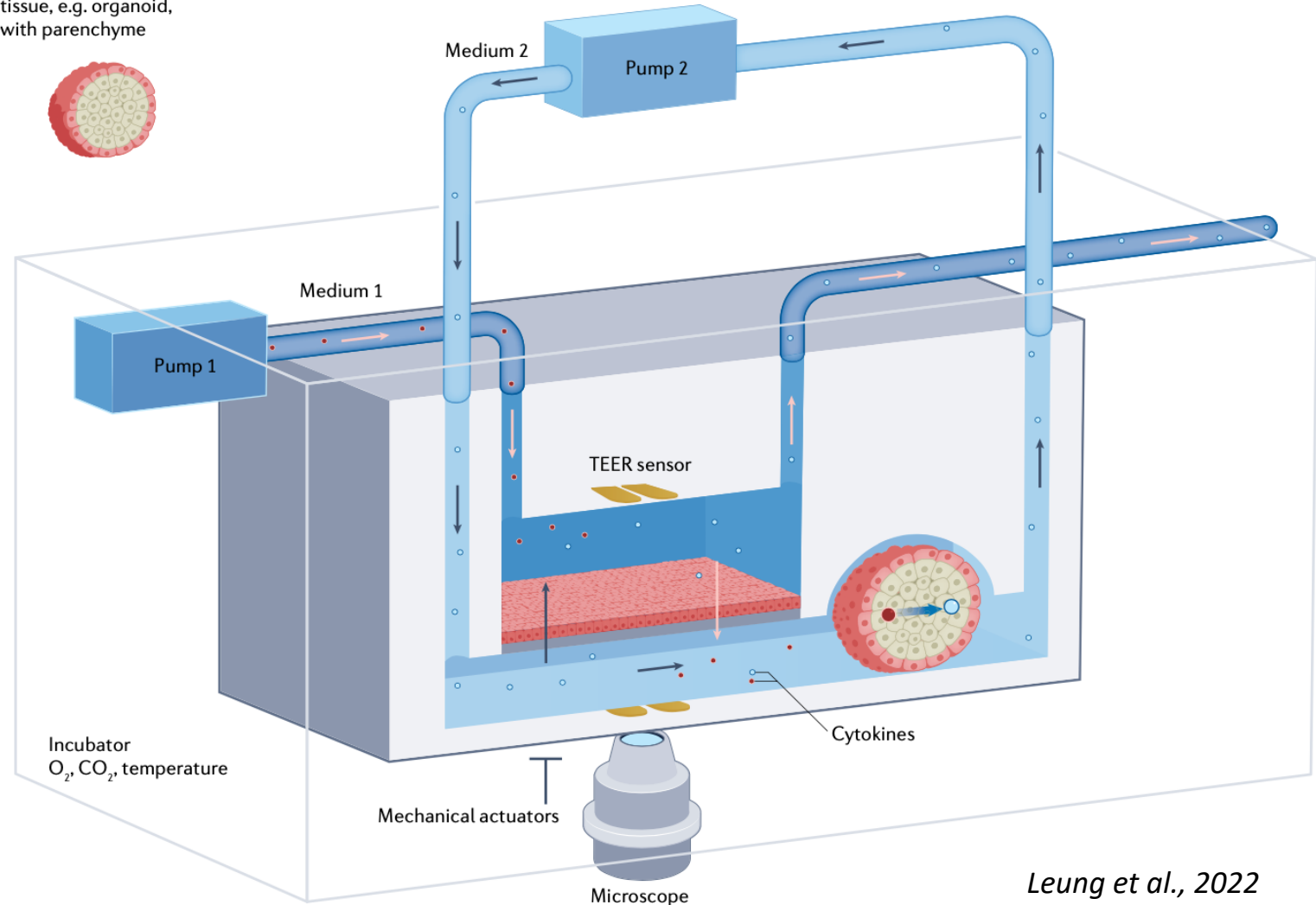


c Selection of biological elements



d Supporting life inside devices

- Perfusion
- Incubators
- Mechanical stimulation
- Controls and sensors



mOoC: Aspects to be considered

- biological scaling
- maintenance of sterility when building or connecting tissue modules
- use of a common medium
- incorporation of bubble traps
- control of varying flow rates

Current models of mOoC

BODY-ON-A-CHIP



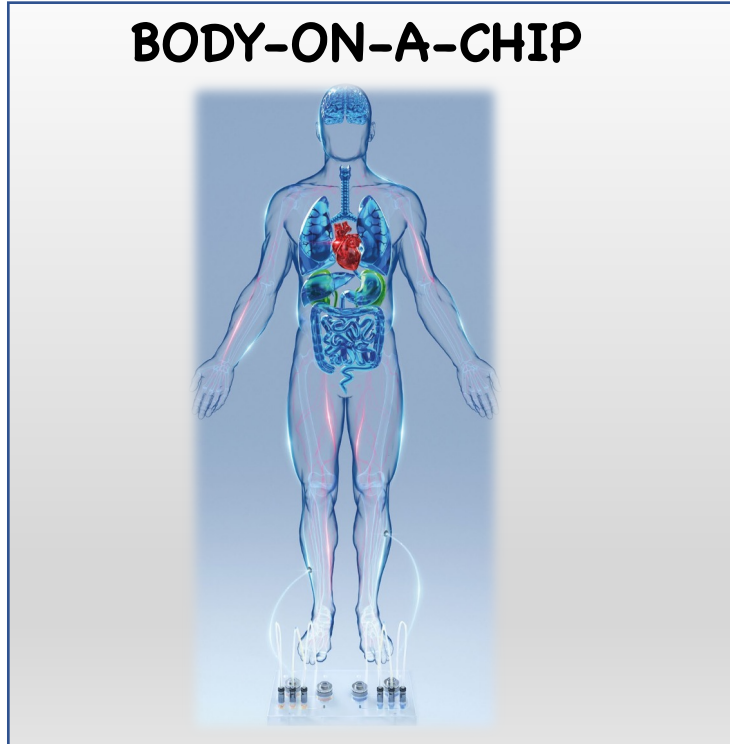
Edington et al., 2018

10-organ 'physiome on a chip' modeling the distribution of in vitro pharmacokinetics and endogenously produced molecules.

Novak et al., 2020

A robotic system maintained the viability and organ-specific functions of eight vascularized, two-channel organ chips (intestine, liver, kidney, heart, lung, skin, blood-brain barrier and brain) for 3 weeks in culture

The Ongoing Challenge for mOoC



However...

A number of organs and tissues are necessarily missing from even the most complex series of linked OoCs, necessitating the need to account for missing organs

?

How can a linked platform model important diurnal or endocrine fluctuations (which affect cell and drug metabolism) if tissues producing or responding to those cues are absent?

A creation of complex engineered 'microformulators' to formulate, deliver and remove culture medium at defined time intervals, simulating the function of missing organs

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