Microfluidic devices

UNIVERSITÀ DEGLI STUDI DI TERAMO
UNITE

Corso di Laurea Magistrale in Biotecnologie Avanzate
AA 2021-2022

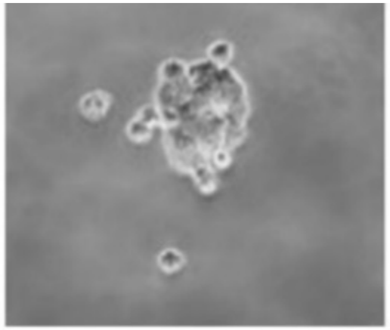
Corso: Tecnologie per la produzione di dispositivi biomimetici (3CFU)

LECTURE 8

Creating environments for in vitro cell growth

cells are grown as monolayers on flat surfaces

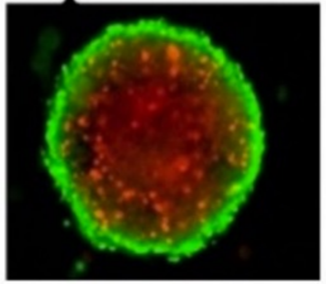
cells grow and interact in all dimensions



Adapted from Singh et al., 2022 and for Tajeddin et al., 2021

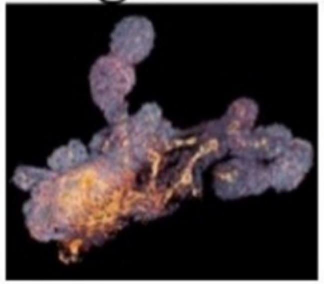
3D

Spheroids



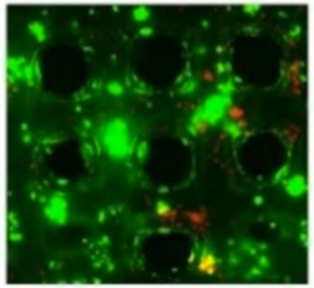
Multicellular, spherical structures composed of aggregated cells that do not adhere to a substrate but adhere to each other

Organoids



A self-organizing 3D cell structure that repre- sents an organ with in vivo-like functions and physiology

Printed Tissues

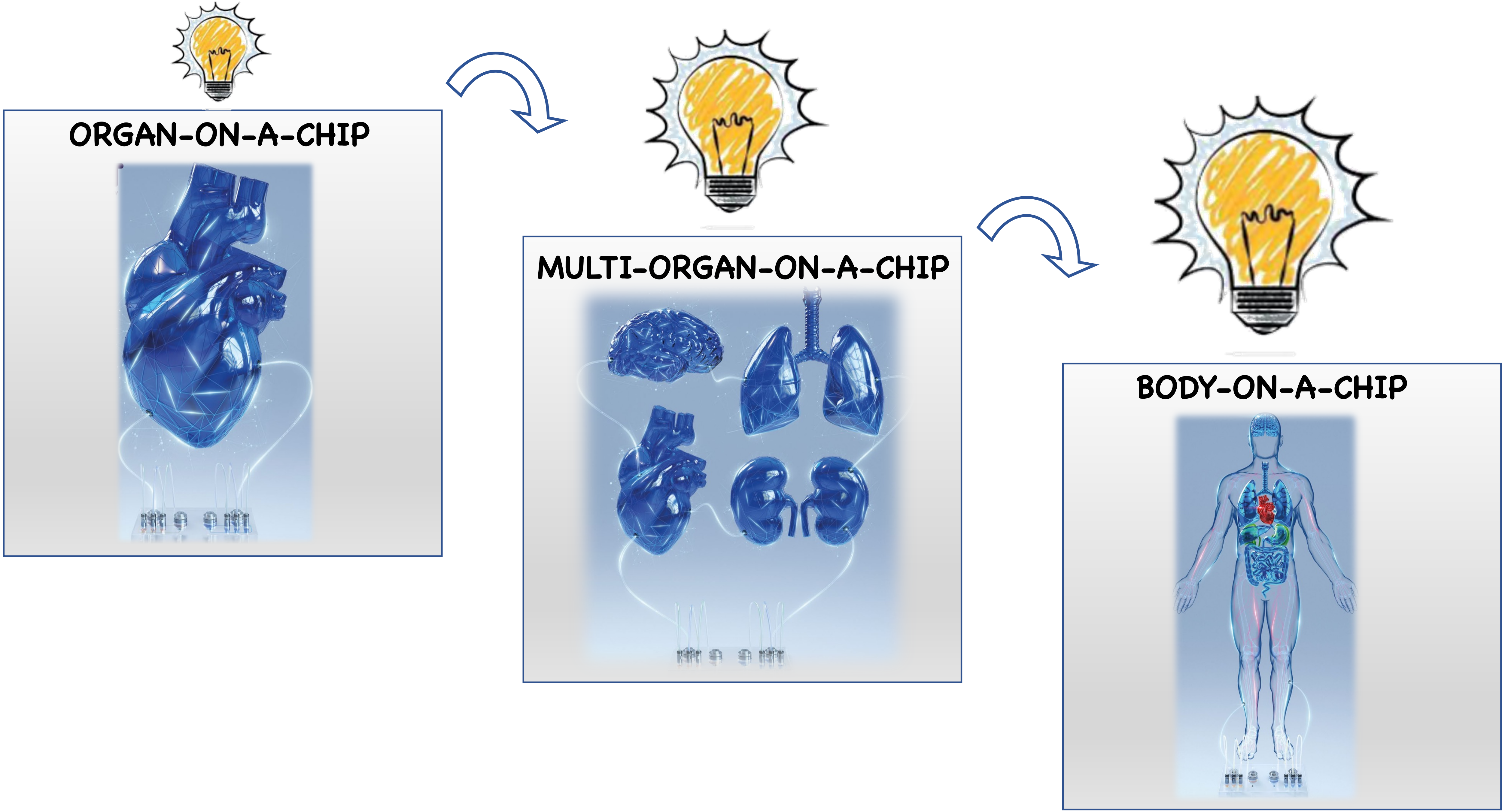


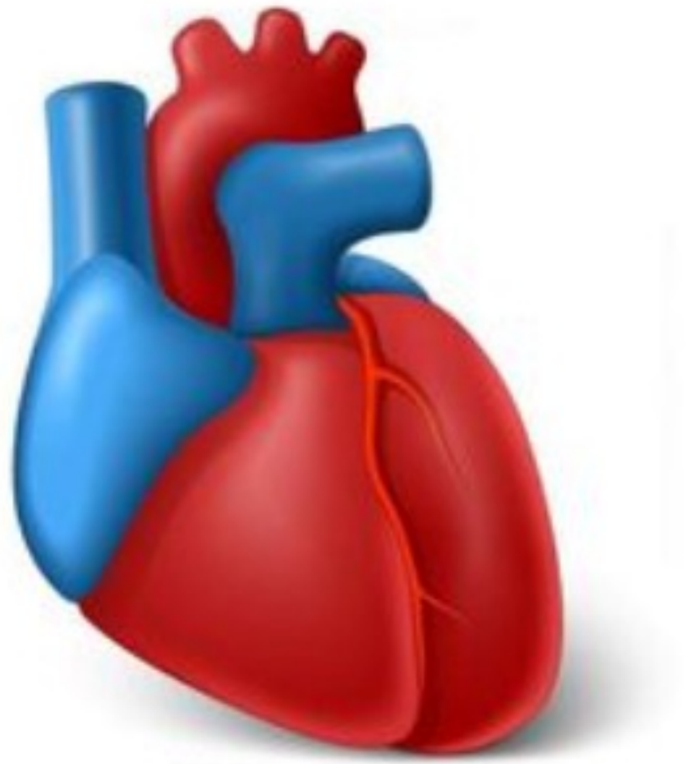
Accurate 3D printed tissues/organs through controlled localization of cells and materials

Microfluidics

Microfluidic devices consisting of multi channels compatible with cell culturing which resembles the physical and physiological functions of a specific organ

Adapted from Tajeddin et al., 2021


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

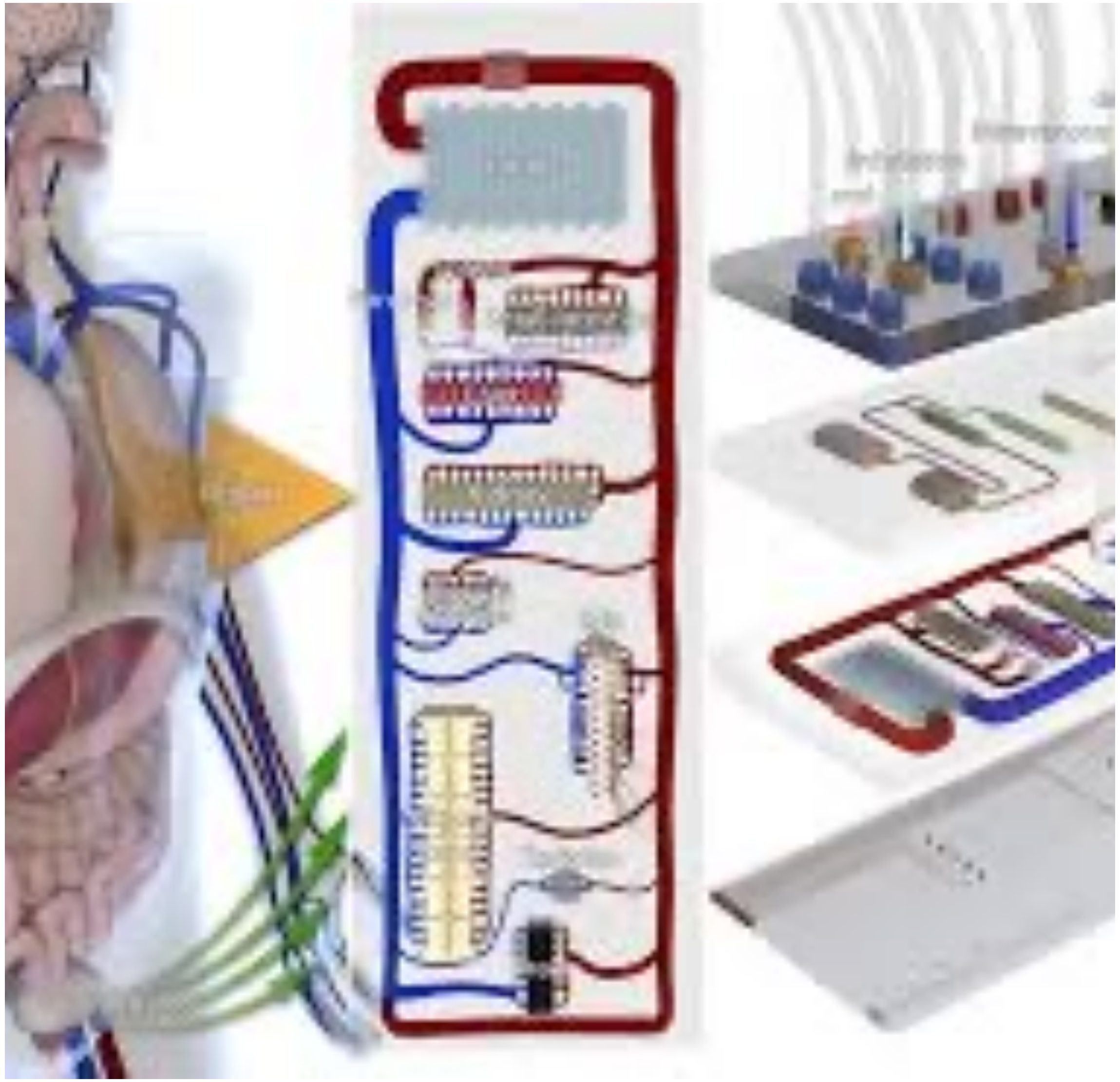
- Platform design
- Platform fabrication

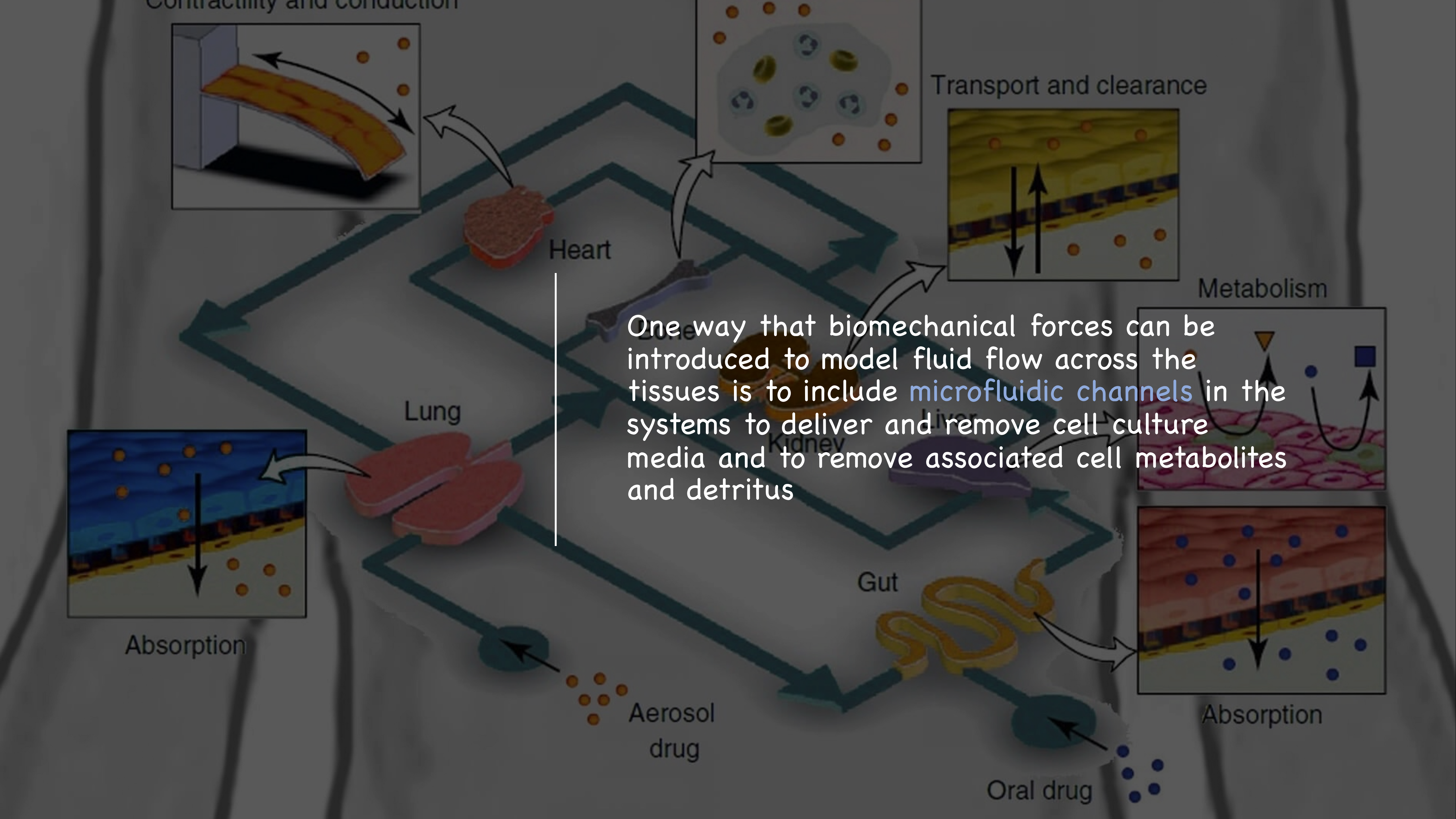
Conceptual OoC design

- 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





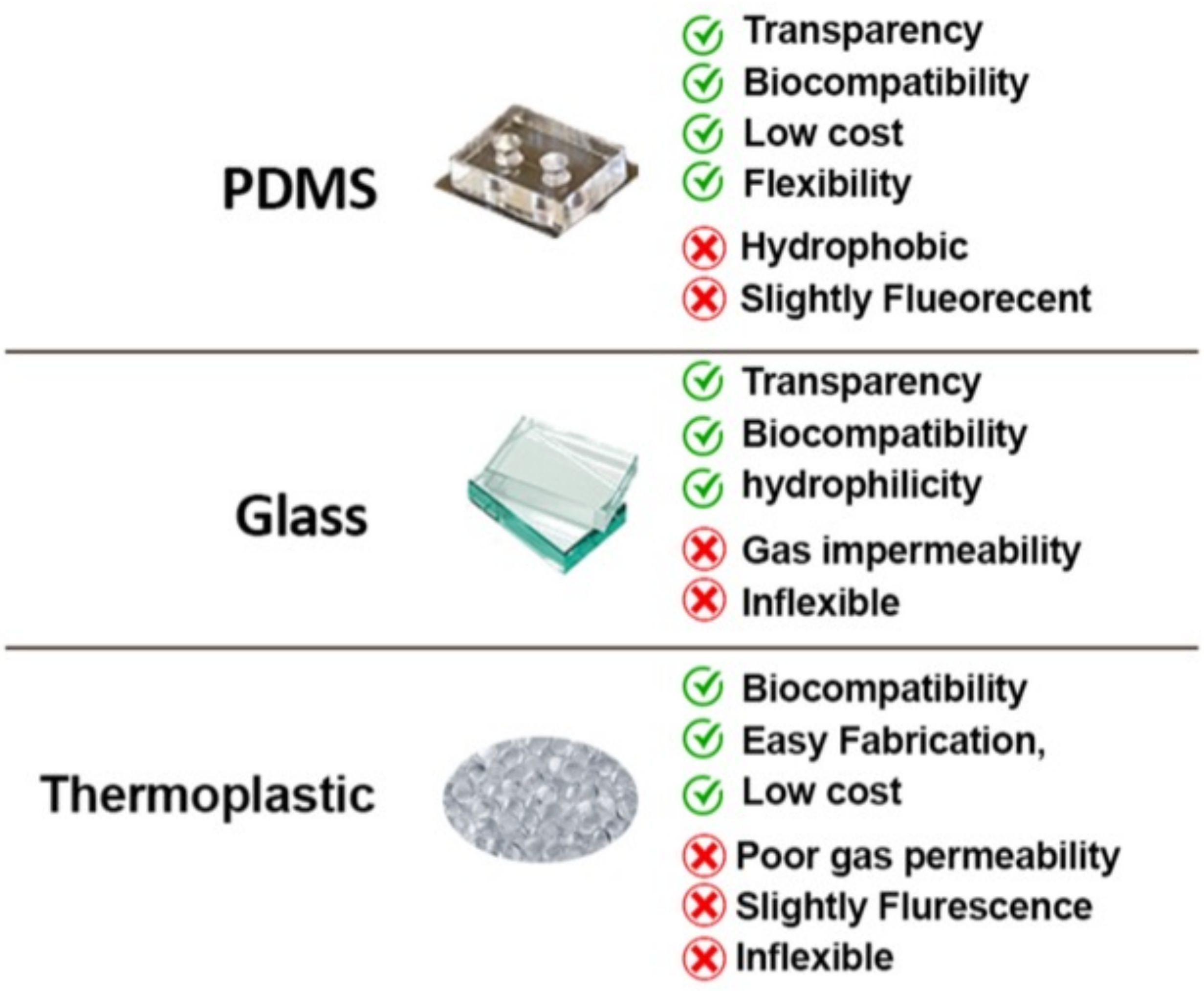
One way that biomechanical forces can be introduced to model fluid flow across the tissues is to include microfluidic channels in the systems to deliver and remove cell culture media and to remove associated cell metabolites and detritus

OoC: Fabrication Materials

Polydimethylsiloxane:
a silicon-based elastomer

soda lime, Quartz, Borosilicate
They are a mixture of silicon dioxide (SiO_2), the base material of glass, with other oxides, such as CaO and MgO

polymethyl methacrylate (PMMA)
or copolymers (COC)



Tajeddin et al., 2021

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

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

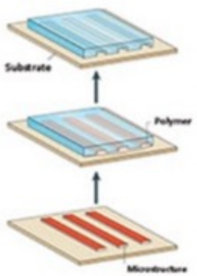
TOP-DOWN

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

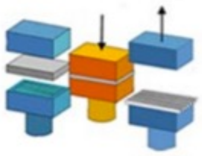
Elastomers



Soft-lithography

- Combination of photolithography and molding
- Suitable for elastomeric materials

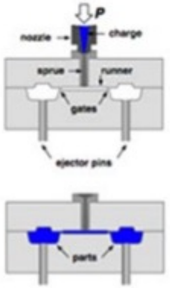
Thermoplastic materials



Hot Embossing

- Requires master mold fabrication
- Suitable for polymeric materials

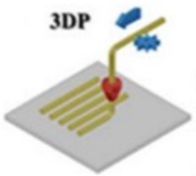
Polymers



Injection Molding

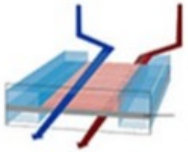
- Requires master mold fabrication
- Low-cost high precision microfabrication suitable for batch production

Elastomers



3D Printing

- Supports both additive and subtractive manufacturring
- 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

Green: Positive; Yellow: Moderate; Red: Negative.

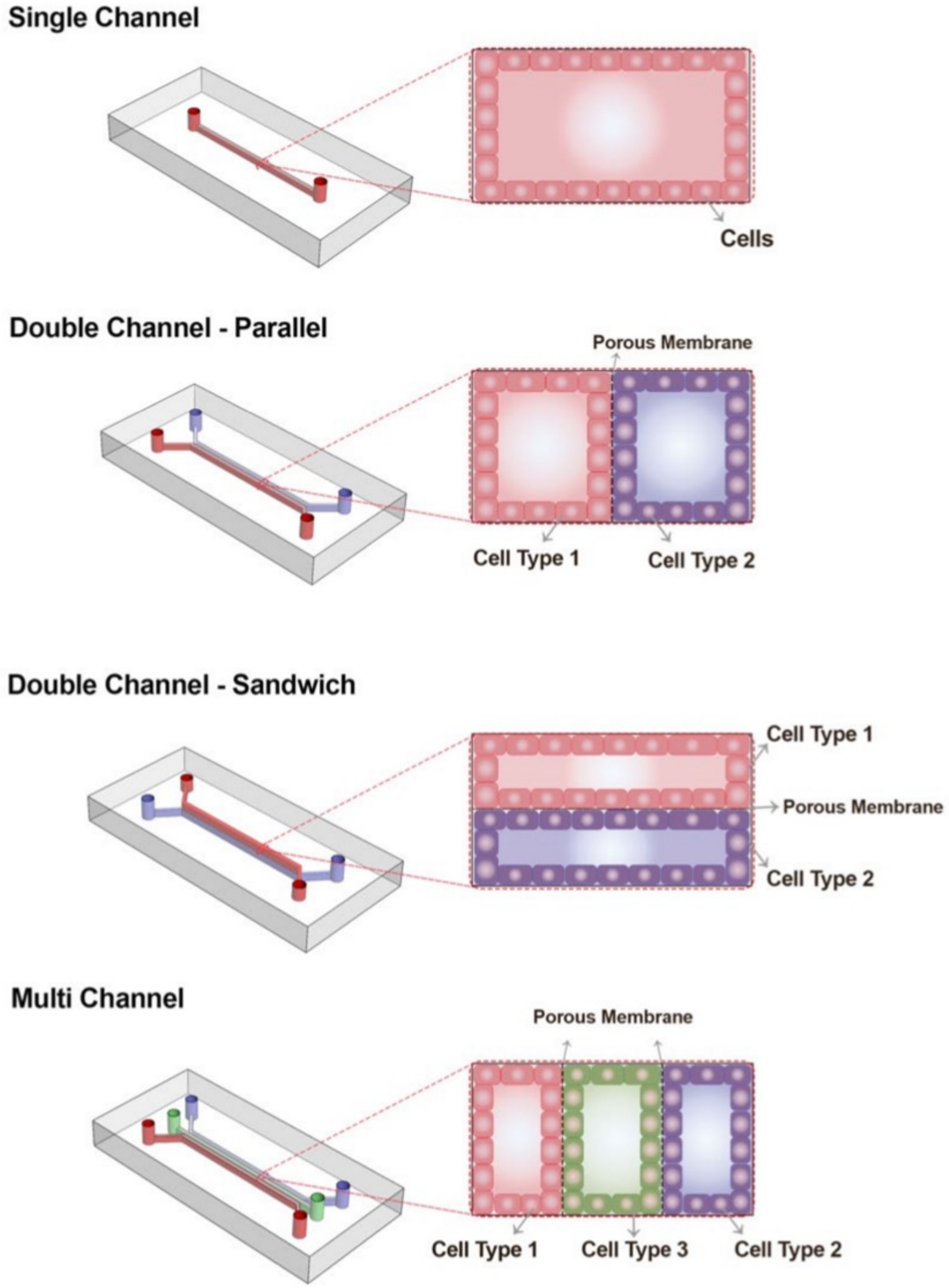
Tajeddin et al., 2021

OoC: Geometry and Dimensions

Classified based on numbers and organization of channels/compartments

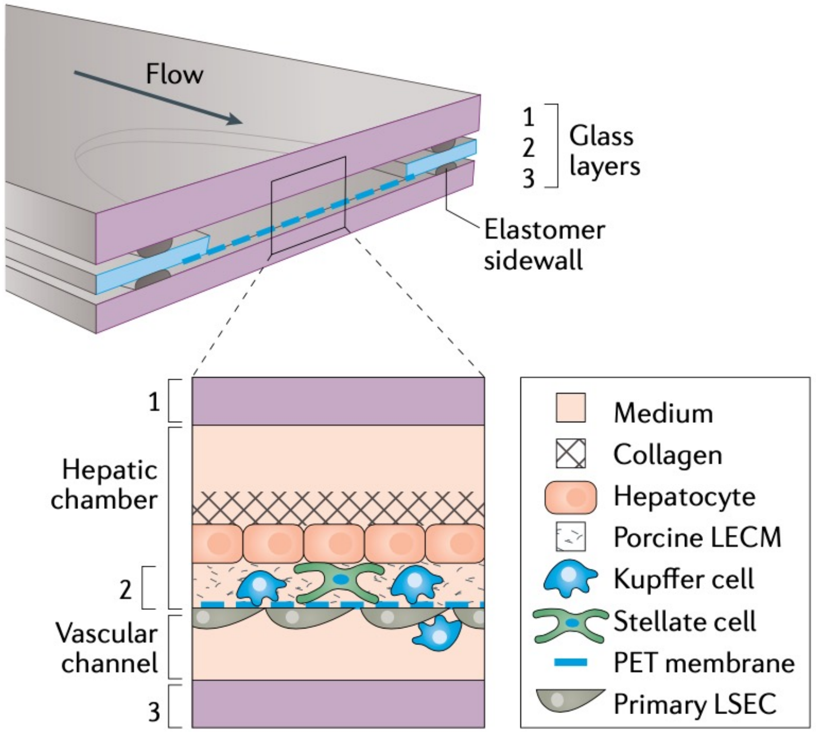
Double channel design is mostly used where one compartment was used to mimic the blood vessels and the other compartment(s) for the actual tissue cells

Porous membranes are usually polymeric

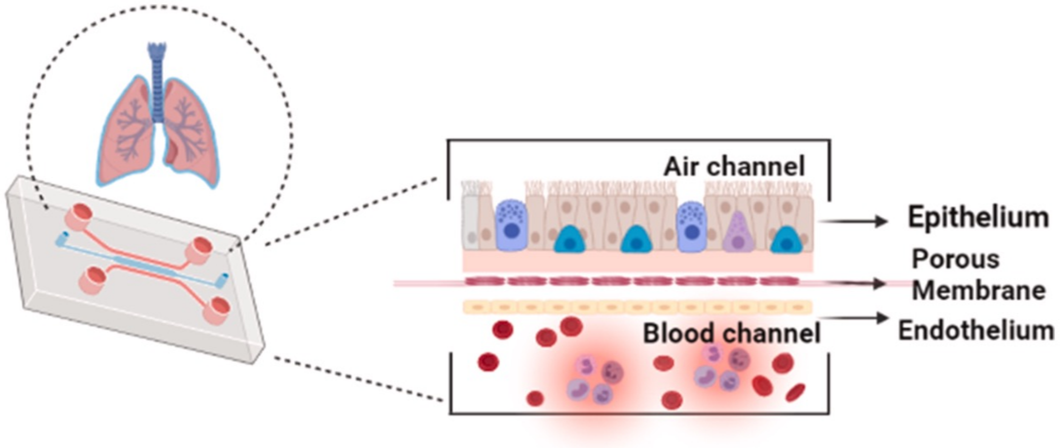


Tajeddin et al., 2021

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

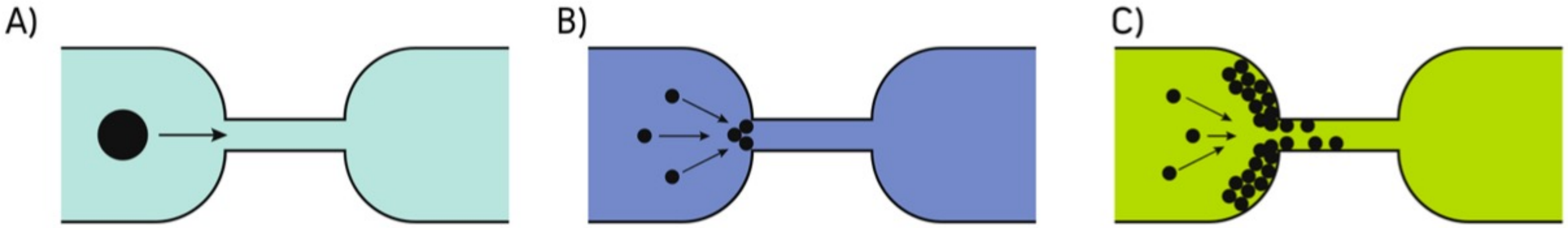


Figure 4. Clogging mechanisms: (**A**) sieving, (**B**) bridging, and (**C**) aggregation.

Tajeddin et al., 2021

SIEVING: Particles are larger than dimention 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: Key features for setting up OoC

TECHNICAL

BIOLOGICAL

Cell source
Cell scaffolds

Understanding tissue composition and scaffold or ECM influence cellular fuctions 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 ceration of isogenic cell lines for genetic disorders

Cell scaffolds

It is importatnt to reconstitute a physiological environment, conductive to cell growth.
The choice of the decellular scaffolds or hydrogels (naturale or synthetic) shuld be tested as funztion of the tissue type

OoC applications

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

TOXICITY
Assessing response to therapeutics with known or unknown mechanisms of action

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 absorbtion, 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 pahse I clinical trials

The use of OoC might allow to overcame 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

OoC: Disease modelling in vitro

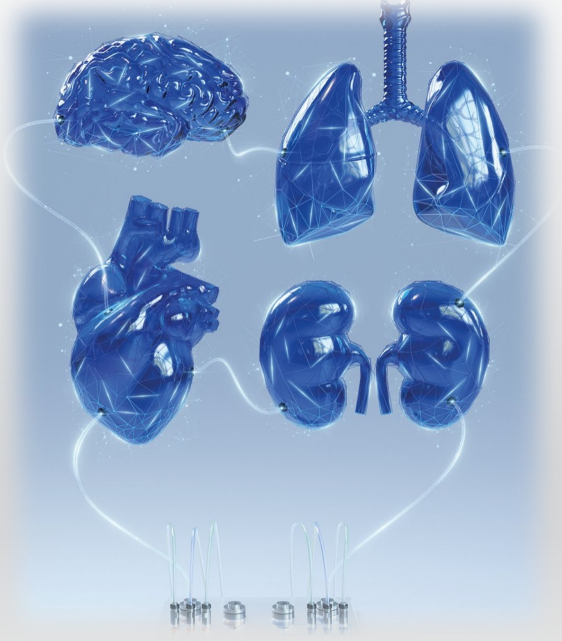
With iPS
Advantages: High plasticity and differentiation potential rendering broad disease modelling application
Limitations: The difficulty to produce and adeguate number of mature, differentiated cells with the necessary purity of many tissues.

With tumor cells
Advantages: accurate modelling
Limitation: low plasticity. Need of the specific cellular model for the targeted tumor

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

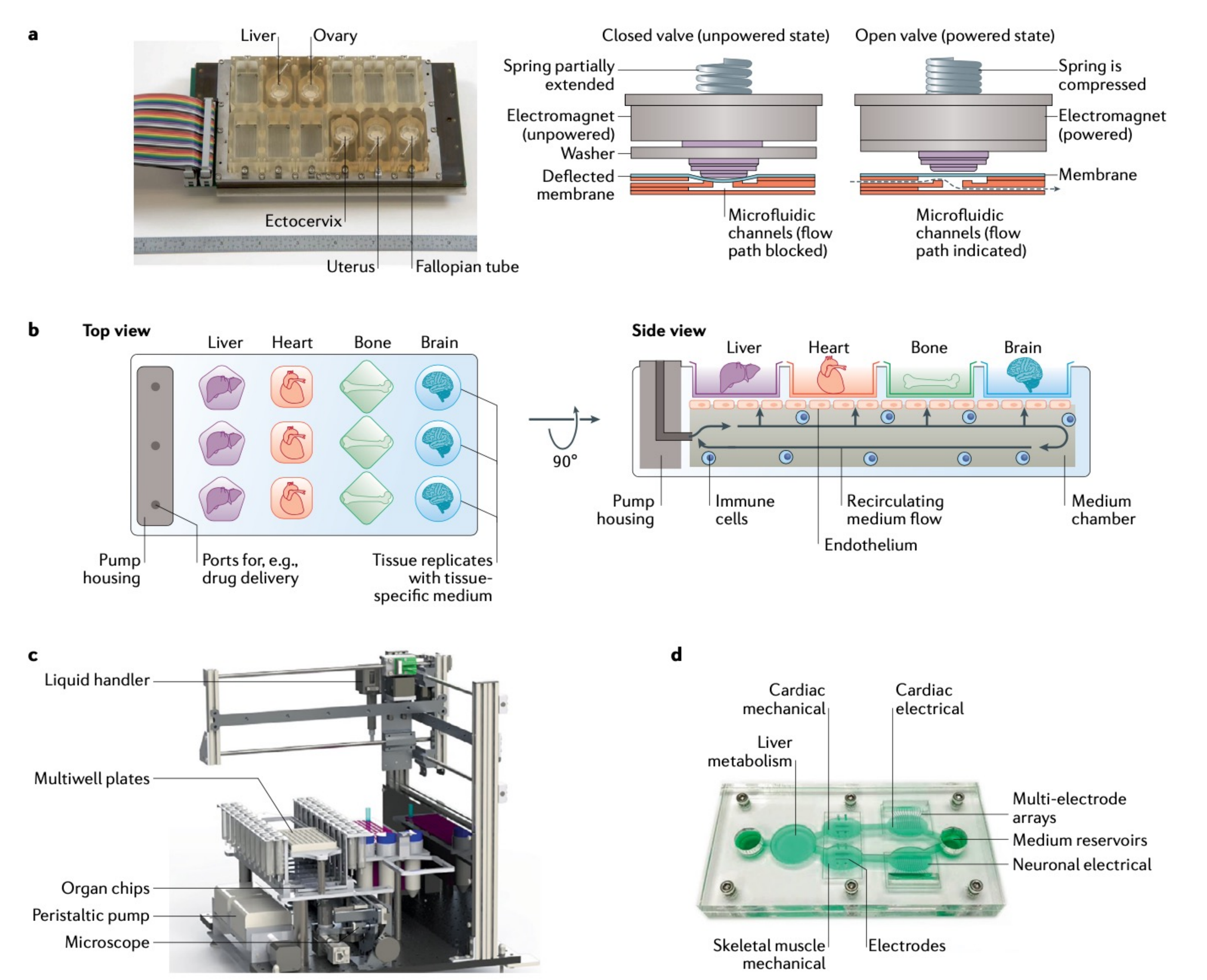
TARGETED Disesease modeling (and also therapy) with chip devices bearing patient-derived primary or iPS cell derivates

MULTI-ORGAN-ON-A-CHIP



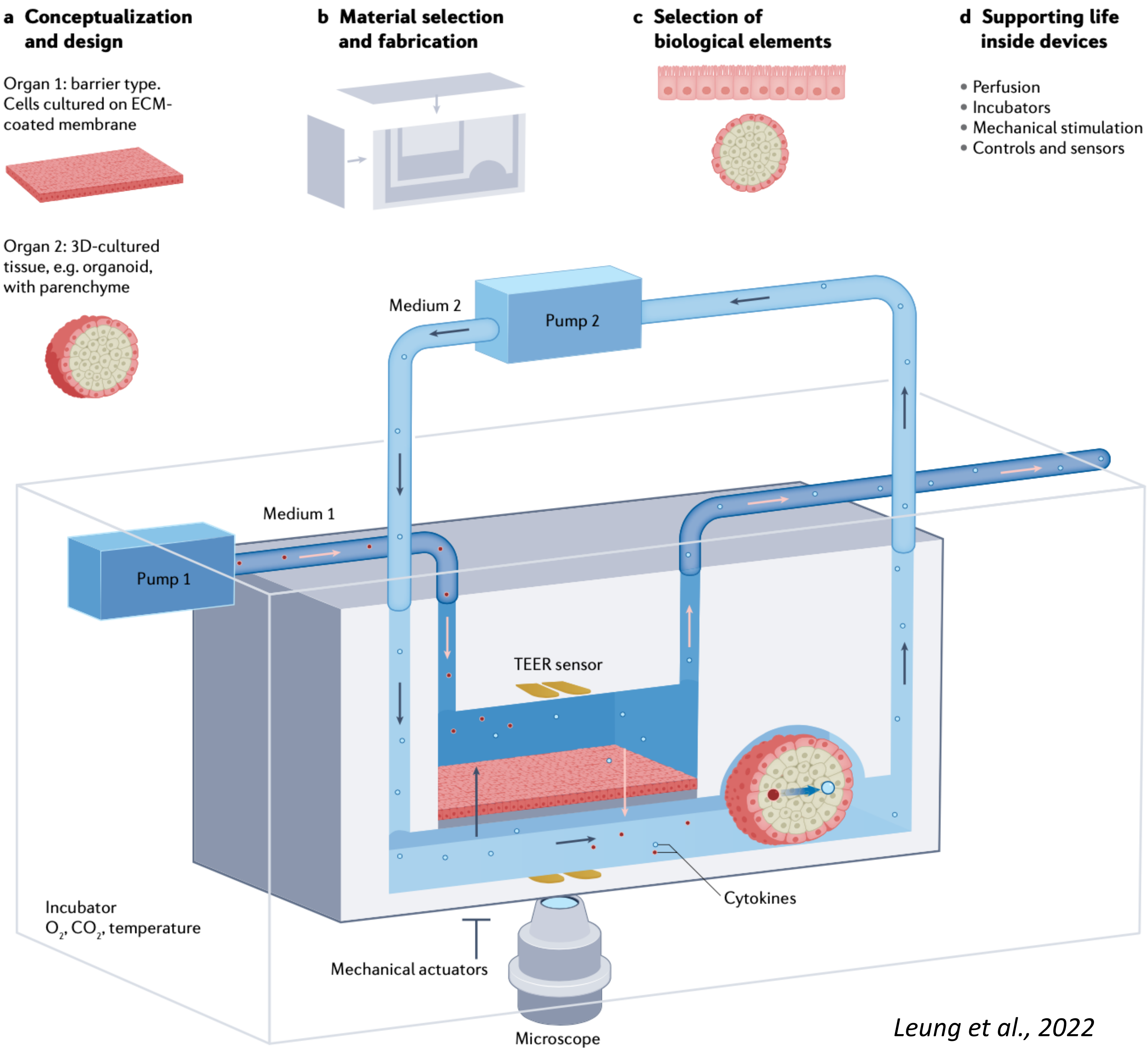
mOoC:

Systems linking multi-organ systems



Low et al., 2020

Example: a generic 2-organ system



Leung et al., 2022

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

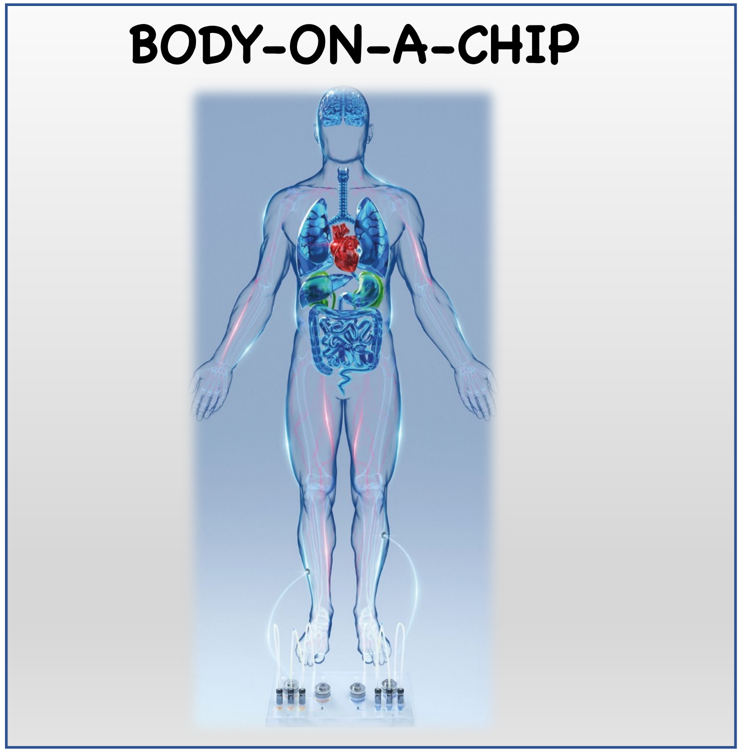
Current models of mOoC

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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