INSTITUTO DE PESQUISAS TECNOLÓGICAS

COMUNICAÇÃO TÉCNICA

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BBB-on-a-chip: a new model to mimic the human blood-brain barrier

Sheila Souza Gomes Fortes

Palestra apresentados na na USP/IQSC, 2024. 33 slides.

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BBB-on-a-chip: a new model to mimic the human blood-brain barrier

Advisor: Prof. Dr. Emanuel Carrilho

Ph.D Student: Sheila Sousa Gomes Fortes

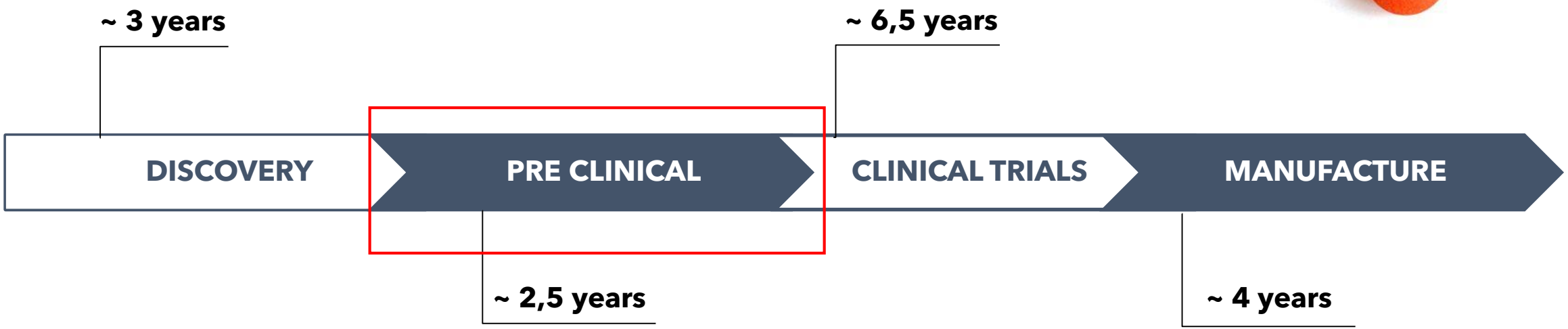
90% new drug developments fail in clinical trials

Who I am

Sheila Sousa Gomes Fortes

- Polymer Technologist – Fatec Mauá (2015)
- MSc in Biomedical Engineering – UFABC (2018)
- Ph.D. Student in Organic and Biological Chemistry at IQSC USP
- Researcher at IPT
- Visiting Graduate Student at Wyss Institute at Harvard University

Development of new drugs



10 - 15 years
2 - 3 billions $

Acta Pharmaceutica Sinica B, Volume 12, Issue 7, July 2022, Pages 3049-3062

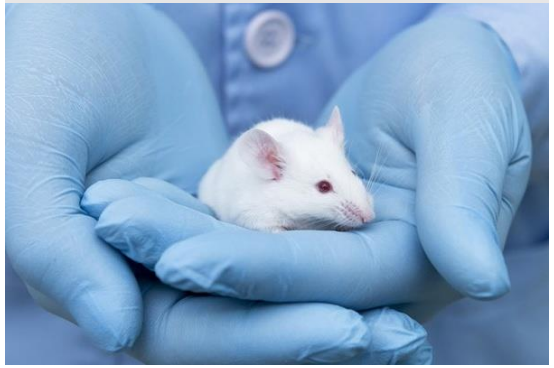
Why do this failures happens?

Current methods used during the pre-clinical trails of new drugs

2D cell culture

- Simple and reproducible
- Low cost
- High Throughput
- Real-time monitoring
- Long-term cell viability
- Patient-specific cells
- No ethical issues

Animal Models

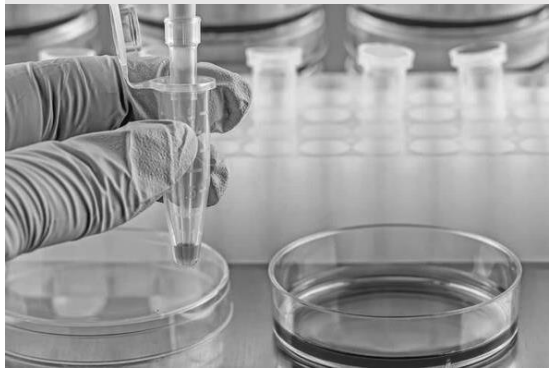


- 3D-tissue architecture
- Immune system
- Hemodynamic system
- Physiological biomechanics and biochemical cues
- Multi tissue/organ interaction

Microfluidics Innovation Center. *LUNG-ON-A-CHIP MODEL PACK* https://microfluidics-innovation-center.com/application-packs/lung-on-a-chip-model/

Current methods used during the pre-clinical trails of new drugs

2D cell culture



- Single cell types
- No physiological biomechanics and biomedical cues
- No hemodynamic system
- Does not mimic 3D tissue architecture

Animal Models

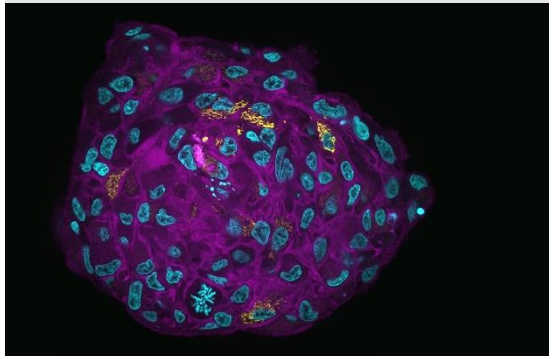
- Expensive
- Time Consuming
- Interspecies variation
- Low-troughput
- Ethical issues
- Findings can be incosistent in translation to human health

Microfluidics Innovation Center. *LUNG-ON-A-CHIP MODEL PACK* https://microfluidics-innovation-center.com/application-packs/lung-on-a-chip-model/

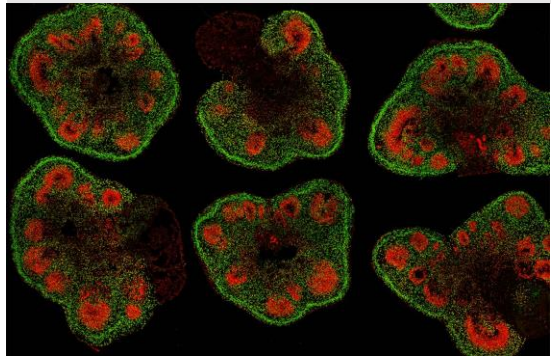
In vitro models for mimicking organ functions

Transwell

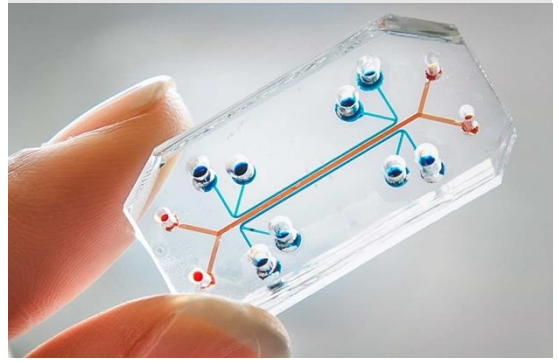
Spheroids



Organoids



Organs-on-a-chip



Microfluidics Innovation Center. *LUNG-ON-A-CHIP MODEL PACK* https://microfluidics-innovation-center.com/application-packs/lung-on-a-chip-model/

Models to mimic organs: transwell

Transwell

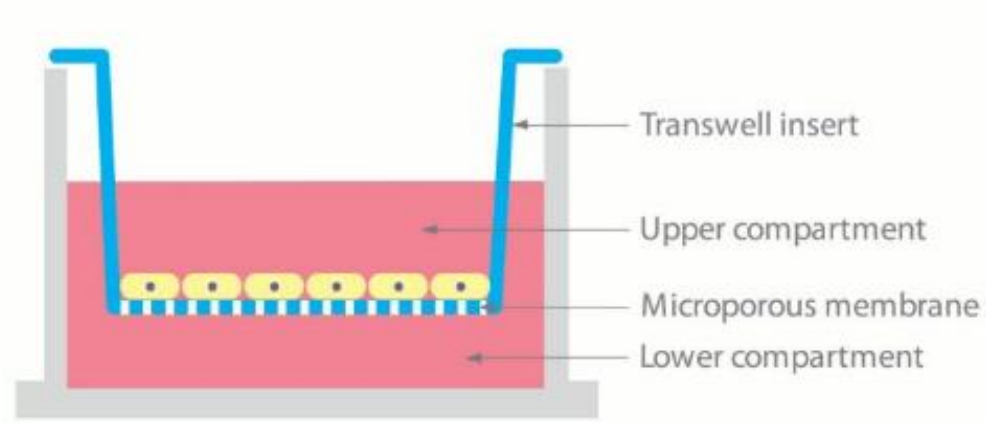
Cell culture system that uses a porous membrane inserted into a well of a culture plate to separate different types of cells or compartments of an experiment

ADVANTAGES

- Simple and reproducible
- Low cost
- Co-culture
- Cell differentiation
- High throughput
- Real-time monitoring
- Long-term cell viability
- Patient-specific cells
- No ethical issues

LIMITATIONS

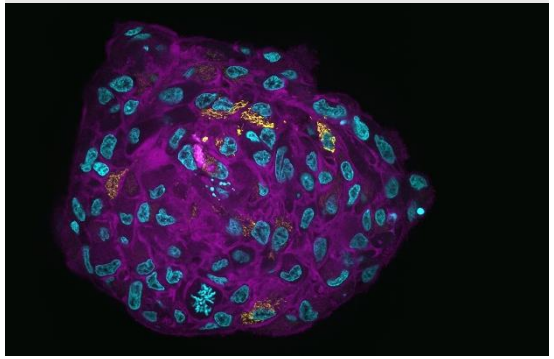
- No physiological biomechamics
- No hemodynamic system
- Does not mimic 3D tissue architecture
- Inadequate nutriente and waste transport



Microfluidics Innovation Center. *LUNG-ON-A-CHIP MODEL PACK* https://microfluidics-innovation-center.com/application-packs/lung-on-a-chip-model/

Models to mimic organs: spheroids

Spheroids



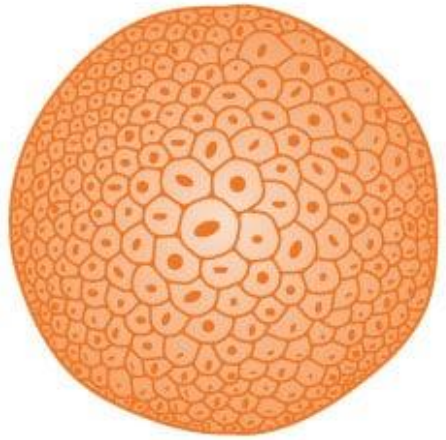
A three-dimensional cluster of cells that forms spontaneously in culture. These structures are often used as more realistic models of tumors or other tissues compared to two-dimensional cultures.

ADVANTAGES

- Simple and round
- Easier to manage and culture in large quantities
- Mimics 3D tissue architecture
- Full cell differentiation
- Cell-cell and cell-ECM interaction presente
- Real-time monitoring

LIMITATIONS

- Limited diversity
- Challenging to maintain over long time
- No physiological biomechamics
- No hemodynamic system
- Does not mimic 3D tissue architecture
- Inadequate nutriente and waste transport

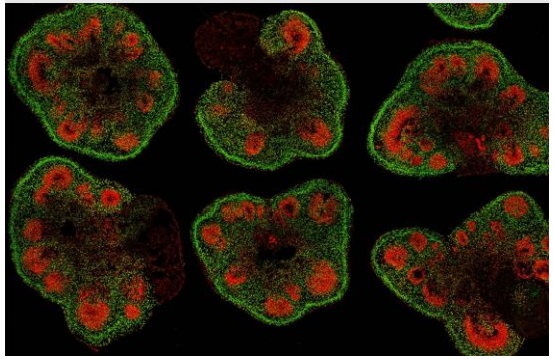


Single cell type and usually from cell lines

Microfluidics Innovation Center. *LUNG-ON-A-CHIP MODEL PACK* https://microfluidics-innovation-center.com/application-packs/lung-on-a-chip-model/

Models to mimic organs: organoids

Organoids



Three-dimensional miniature of an organ grown from stem cells. Organoids replicate some of the characteristics and functions of real organs.

ADVANTAGES

- Phenotypical/physiological relevance
- Mimic the diversity of organs
- Mimics 3D tissue architecture
- Full cell differentiation
- Cell-cell and cell-ECM interaction presente
- Real-time monitoring
- No ethical issues

LIMITATIONS

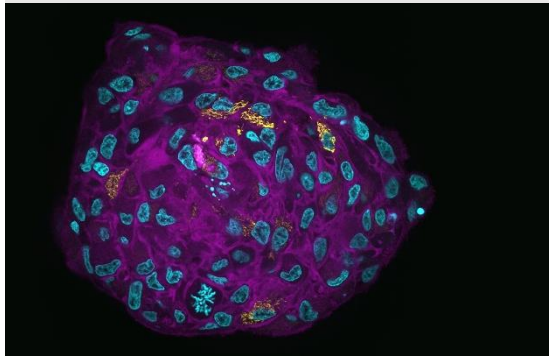
- Lacks imune system
- Multiple tissue/organ interface absent
- Lacks hemodynamic system
- Inadequate nutriente and waste transport
- No standard protocols

Usually stem cell-derived mixture of diferente cell types

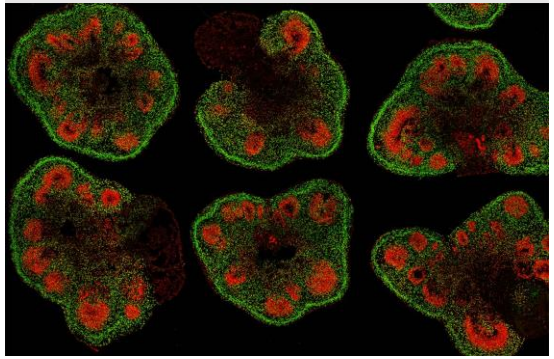
Microfluidics Innovation Center. *LUNG-ON-A-CHIP MODEL PACK* https://microfluidics-innovation-center.com/application-packs/lung-on-a-chip-model/

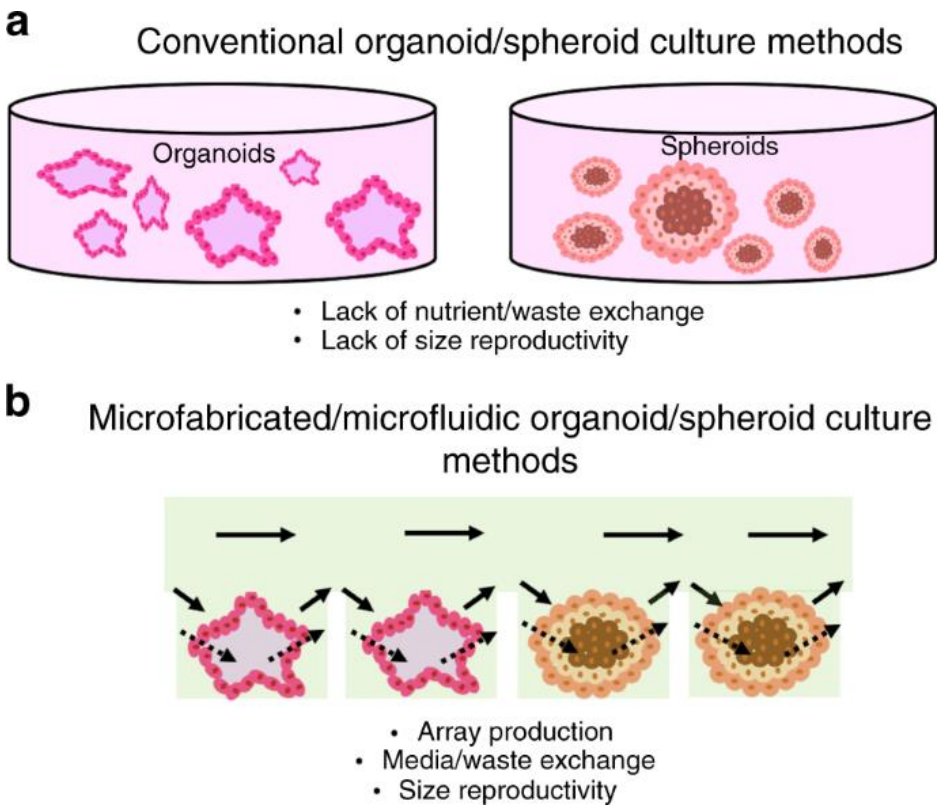
3D models to mimic organs: spheroids and organoids

Spheroids



Organoids

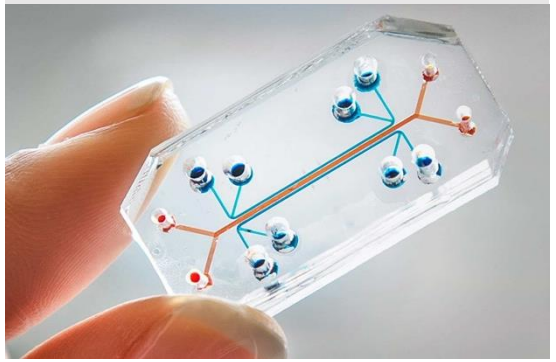




Velasco, V., Shariati, S. A. & Esfandyarpour, R. Microtechnology-based methods for organoid models. *Microsystems Nanoeng.* **6**, (2020).

3D models to mimic organs: organ on a chip

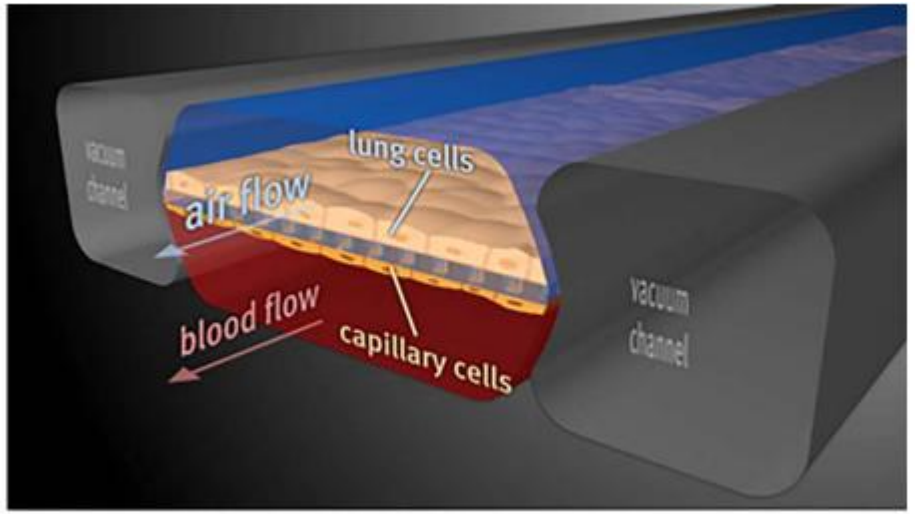
Organs-on-a-chip



Microfluidic device that simulates the physiology and microarchitecture of human organs.

ADVANTAGES

- 3-D tissue architecture
- Controlled microenvironment
- Immune system
- Hemodynamic system
- Physiological biomechanics and biochemical cues
- Multi tissue/organ interaction
- Patient specific cells
- No ethical issues

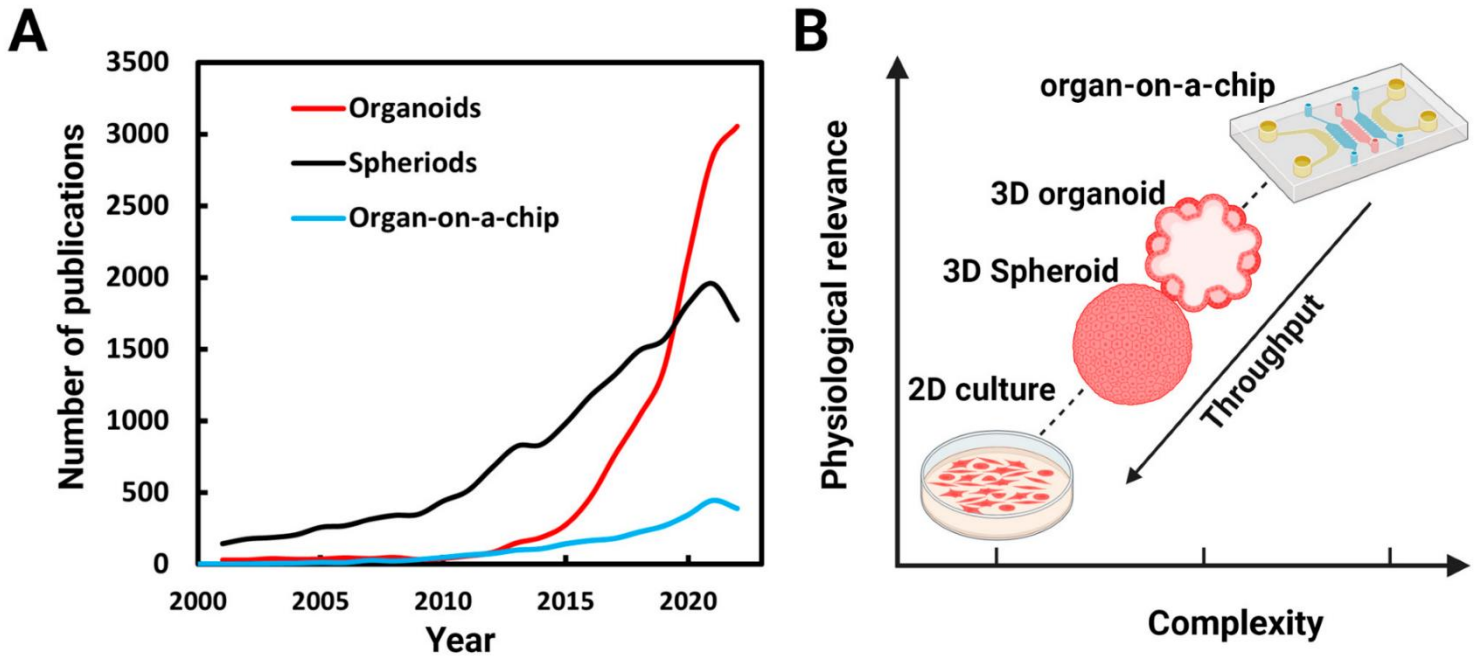


LIMITATIONS

- No standard protocols
- Difficult to scale up
- Complex requiring adroit users

Microfluidics Innovation Center. *LUNG-ON-A-CHIP MODEL PACK* https://microfluidics-innovation-center.com/application-packs/lung-on-a-chip-model/

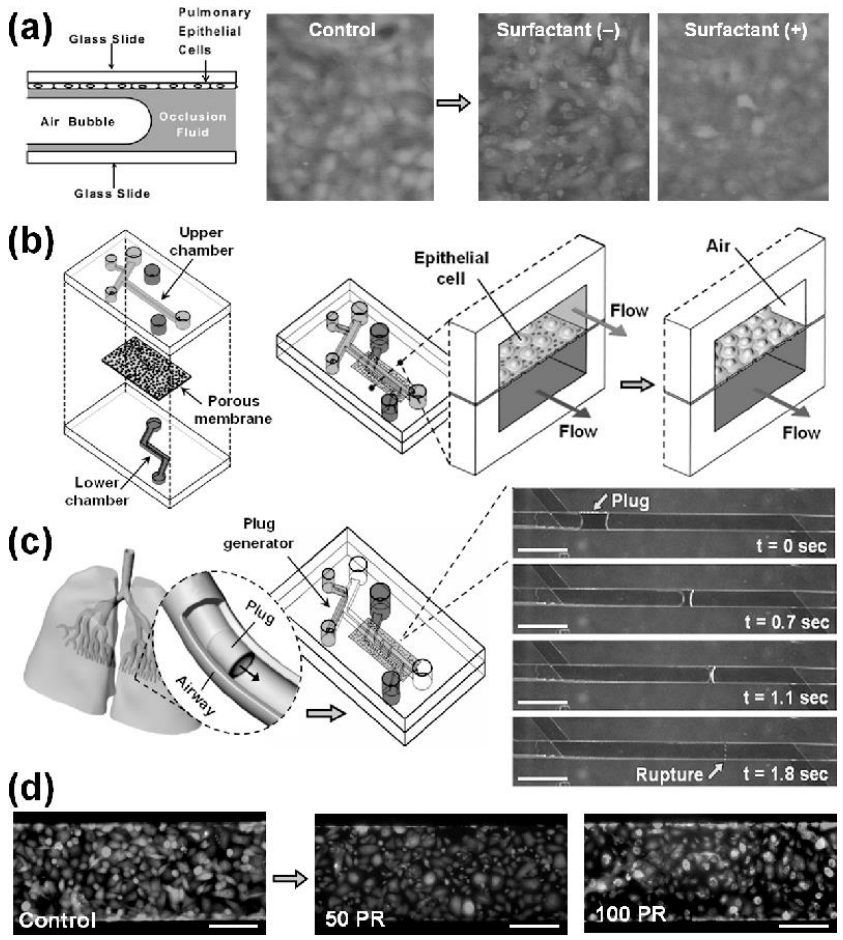
3D models to mimic organs



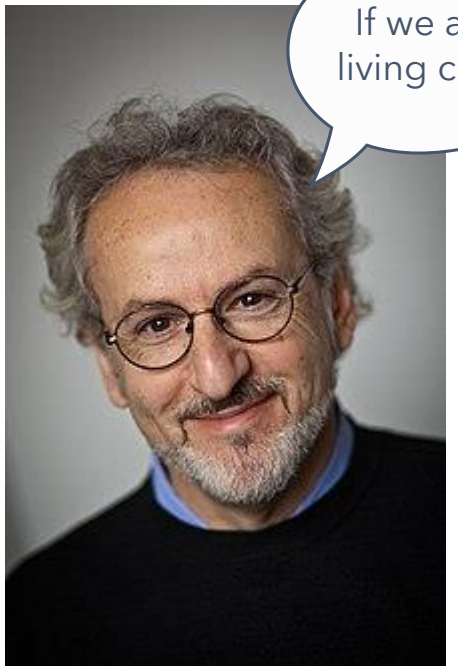
Numbers of publications on organoids, spheroids, and organs-on-a-chip: (A) Number of publications per year found identified by a PubMed search using the terms organoids, spheroids (spheroids and cell aggregates), and organ-on-a-chip between 2001 and 2022. (B) As we get closer to in vivo conditions, the complexities of the systems increases and throughput decreases.

Yousafzai, M. S. & Hammer, J. A. Using Biosensors to Study Organoids, Spheroids and Organs-on-a-Chip: A Mechanobiology Perspective. *Biosensors* **13**, (2023).

Organ-on-a-chip: brief history



Dongeun (Dan) Huh, Ph.D.

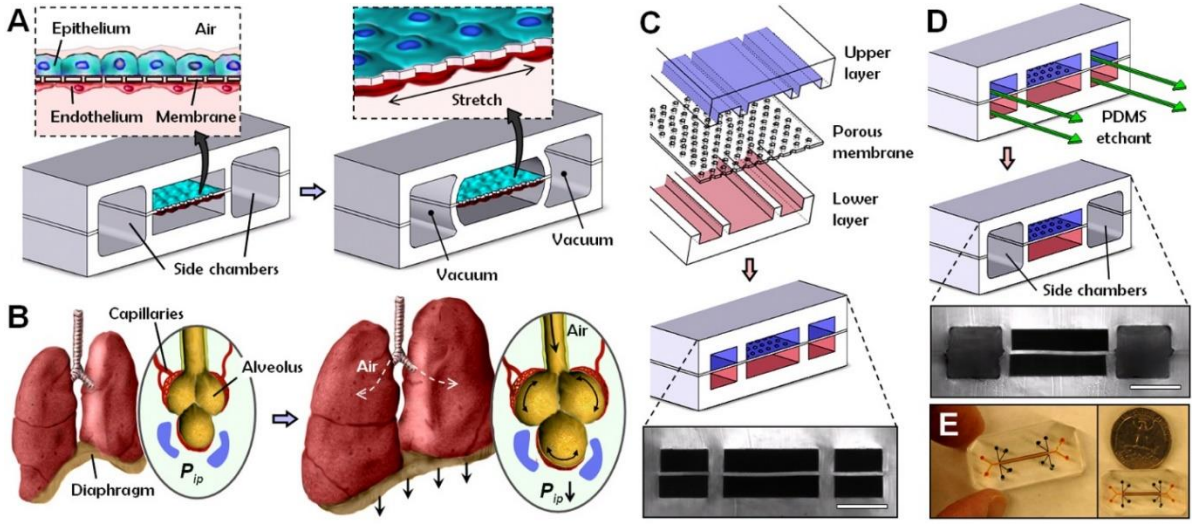


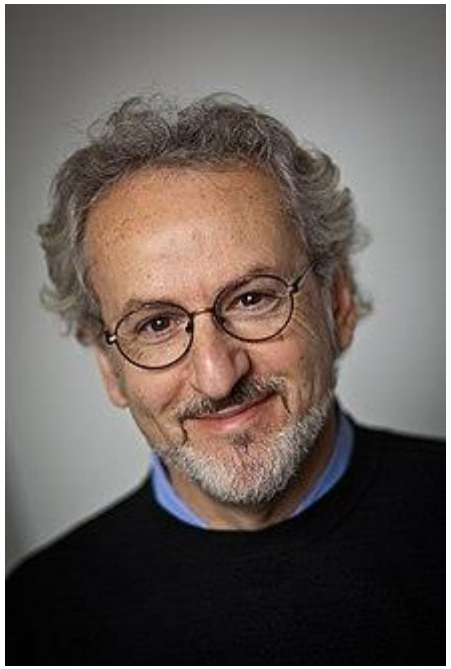
Donald E. Ingber, M.D., Ph.D.

In vitro models of airway reopening: (a) Reopening of closed airway is modeled by the steady progression of a semi-infinite air finger through a liquid-filled parallel-plate chamber lined with pulmonary epithelial cells. The movement of an air-liquid interface at the bubble front results in cellular injury, which can be prevented by pulmonary surfactant. Green and red show live and dead cells, respectively. (Source: [85], reproduced with permission.) (b) Compartmentalized three-dimensional microfluidic small airway system created by soft-lithography-based microfabrication. The polymeric channel system enables proliferation and air-liquid-interfaceinduced differentiation of primary airway epithelial cells in a biomimetic culture environment. (c) More realistic in vitro re-creation of airway reopening is achieved by a computerized air-liquid two-phase microfluidic system integrated on-chip with microfluidic cell culture. This system produces propagation and rupture of a liquid plug with finite lengths by dynamically switching air-liquid two-phase flows in a plug generator. (d) Plug propagation and rupture cause injury of small airway epithelial cells in a dose-dependent fashion. PR represents propagation and rupture. Scale bars = 150 μm.

D. Huh, Y. Kamotani, J. Grotberg, S. Takayama. Engineering Pulmonary Epithelia and Their Mechanical Microenvironments. Engineering, Medicine (2007)

Organ-on-a-chip: brief history





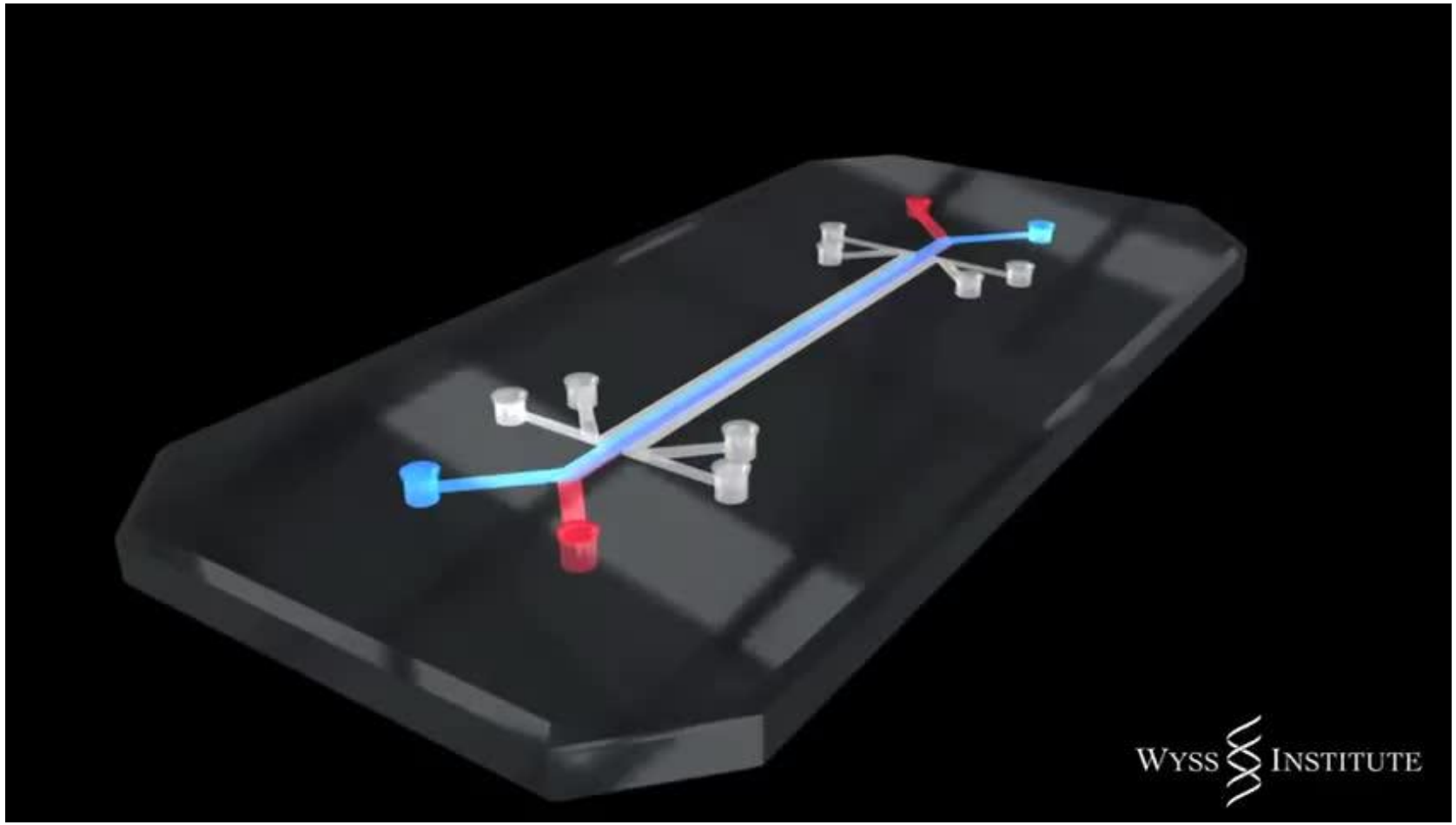
Donald E. Ingber, M.D., Ph.D.

Dongeun (Dan) Huh, Ph.D.

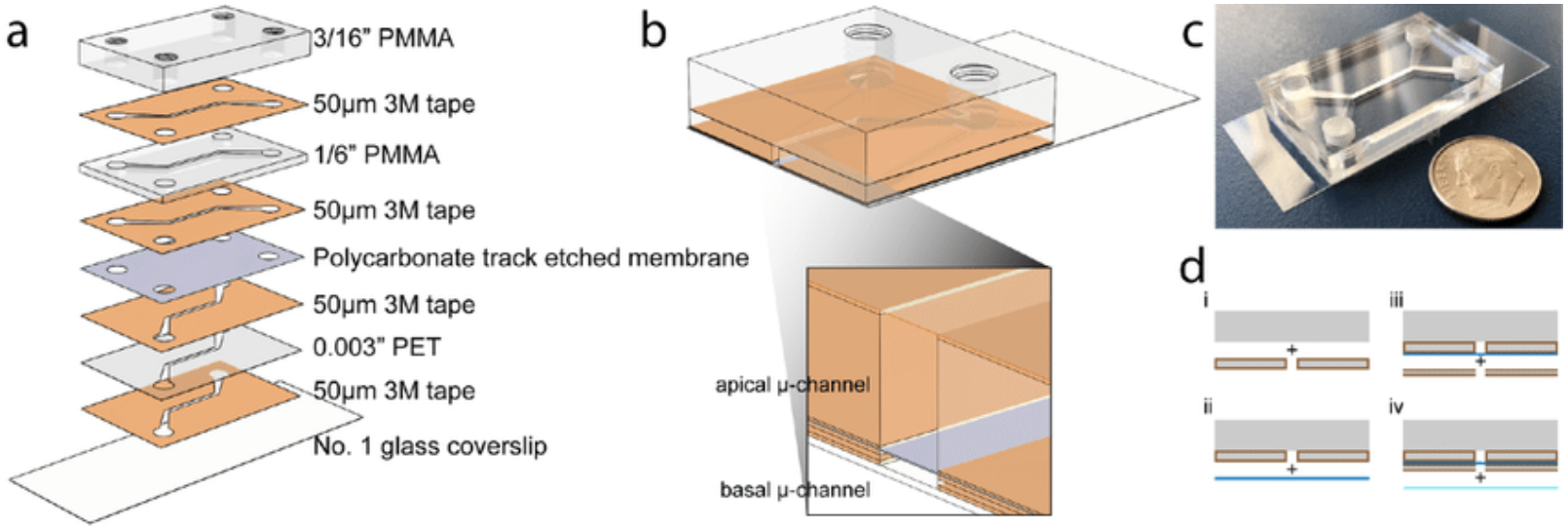
Biologically inspired design of a human breathing lung-on-a-chip microdevice. (A) The microfabricated lung mimic device uses compartmentalized PDMS microchannels to form an alveolar-capillary barrier on a thin, porous, flexible PDMS membrane coated with ECM. The device recreates physiological breathing movements by applying vacuum to the side chambers and causing mechanical stretching of the PDMS membrane forming the alveolarcapillary barrier. (B) During inhalation in the living lung, contraction of the diaphragm causes a reduction in intrapleural pressure (Pip), leading to distension of the alveoli and physical stretching of the alveolar-capillary interface. (C) Three PDMS layers are aligned and irreversibly bonded to form two sets of three parallel microchannels separated by a 10-μm- thick PDMS membrane containing an array of through-holes with an effective diameter of 10 μm. Scale bar, 200 μm. (D) After permanent bonding, PDMS etchant is flowed through the side channels. Selective etching of the membrane layers in these channels produces two large side chambers to which vacuum is applied to cause mechanical stretching. Scale bar, 200 μm. (E) Images of an actual lung-on-a-chip microfluidic device viewed from above.

Huh, D. *et al.* Reconstituting organ-level lung functions on a chip. *Science (80-.).* **328**, 1662–1668 (2010).

How it works:



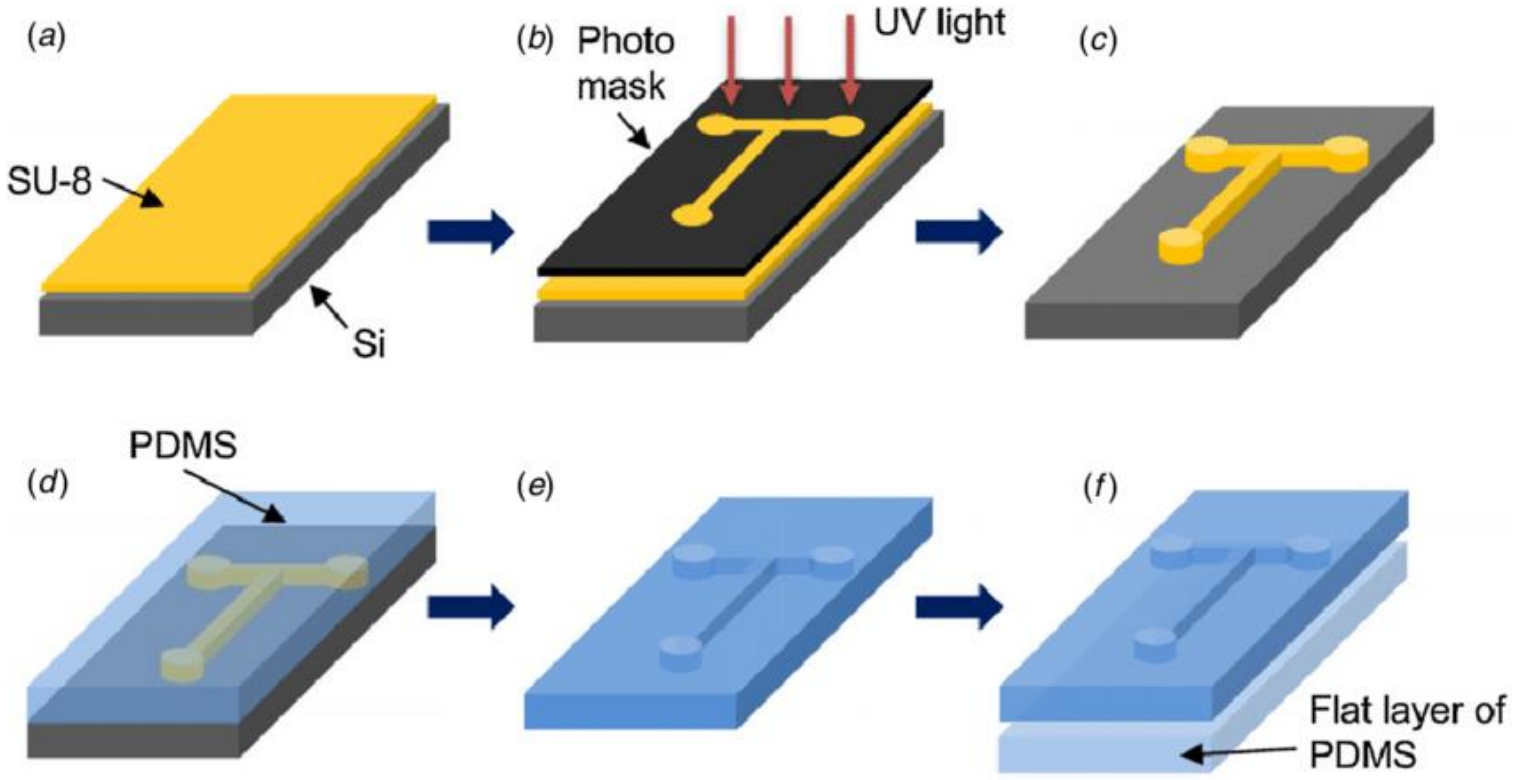
Manufacturing technique: laser cutting



Laser cut and assembled membrane integrated bi-layered organ chip. a) A schematic showing the integration of 9 discrete layers to form a bi-layered organ chip. b) A schematic of an assembly cutaway view of the bi-layered organ chip showing the inner apical and basal microchannels. c) A photograph of the bi-layered organ chip composed of clear PMMA, acrylic adhesive, polycarbonate track etched membrane, and glass coverslip. d) 5 layers are aligned and irreversibly bonded in 4 steps to form the apical and basal channels separated by a polycarbonate track etched membrane with a pore diameter of 1.0 um.

Hosic, S. *et al.* Rapid prototyping of a multilayer microphysiological system for primary human intestinal epithelial culture. *bioRxiv* 400721 (2018).

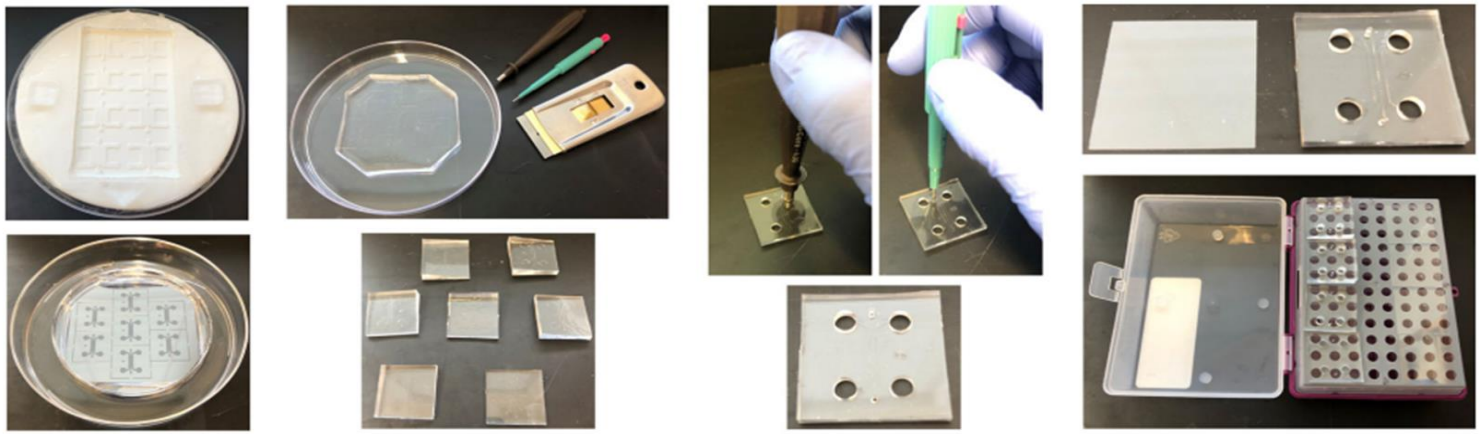
Manufacturing technique: soft litography



A schematic diagram showing the fabrication steps of a PDMS microchannel: (a)-(c) mold fabrication and (d)-(f) replication/bonding processes.

Lee, J. *et al.* Effect of thermal treatment on the chemical resistance of polydimethylsiloxane for microfluidic devices. *J. Micromechanics Microengineering* **23**, (2013).

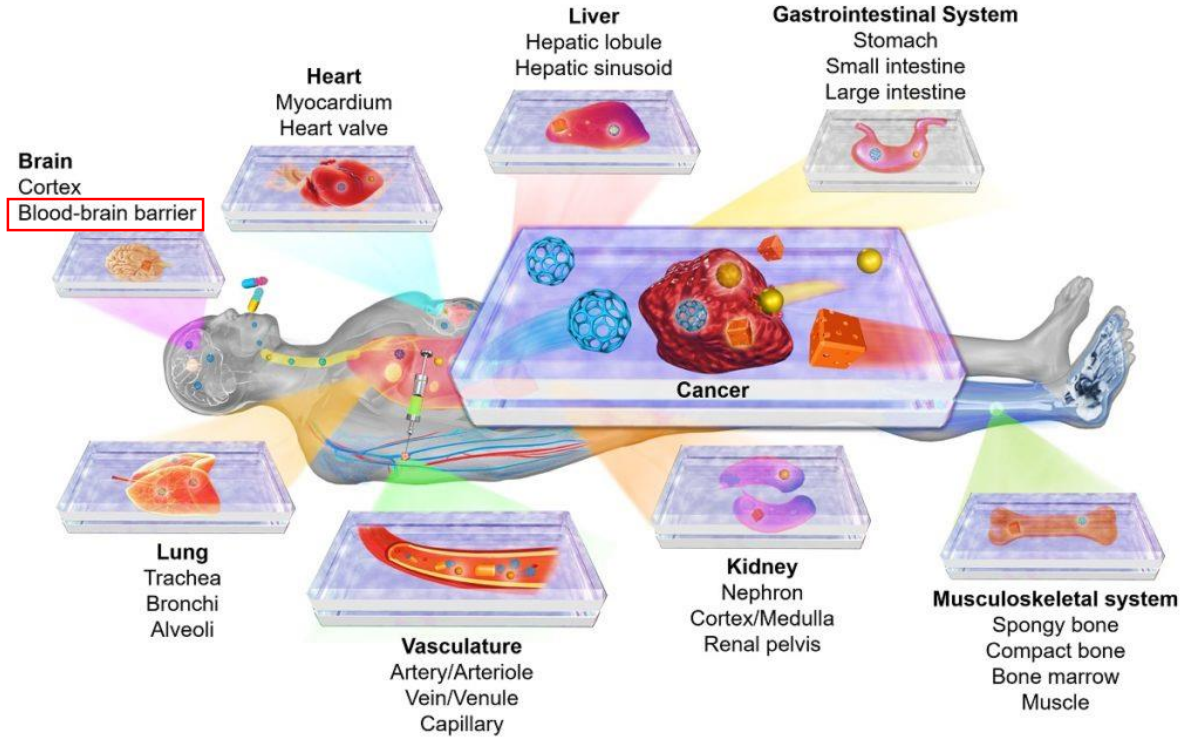
Manufacturing technique: 3D printing

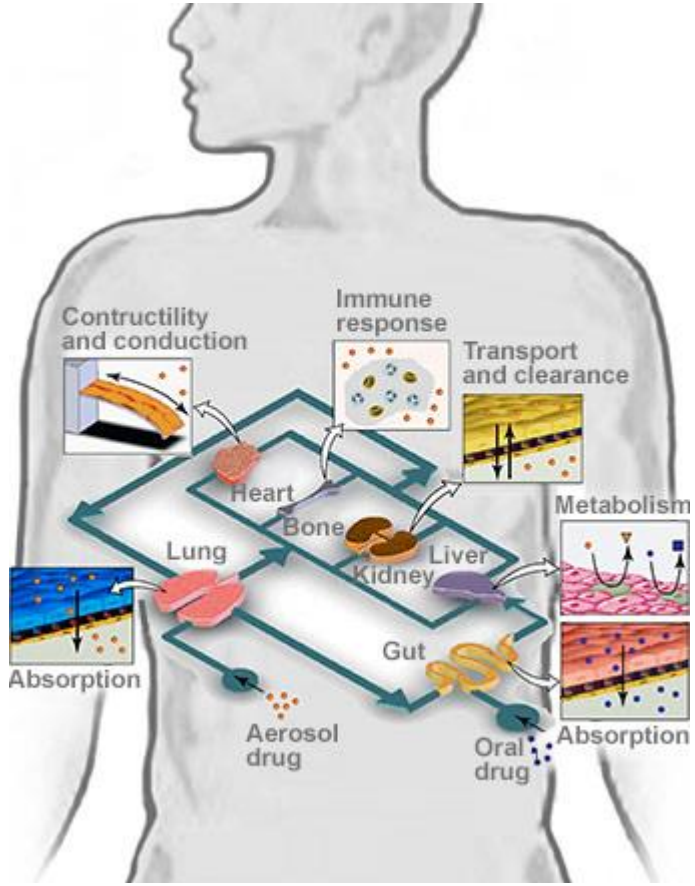


Summary of protocol steps for the fabrication of the macrodevice with a 3D printed mold

Hajal, C. *et al. Engineered human blood–brain barrier microfluidic model for vascular permeability analyses*. *Nature Protocols* vol. 17 (Springer US, 2022).

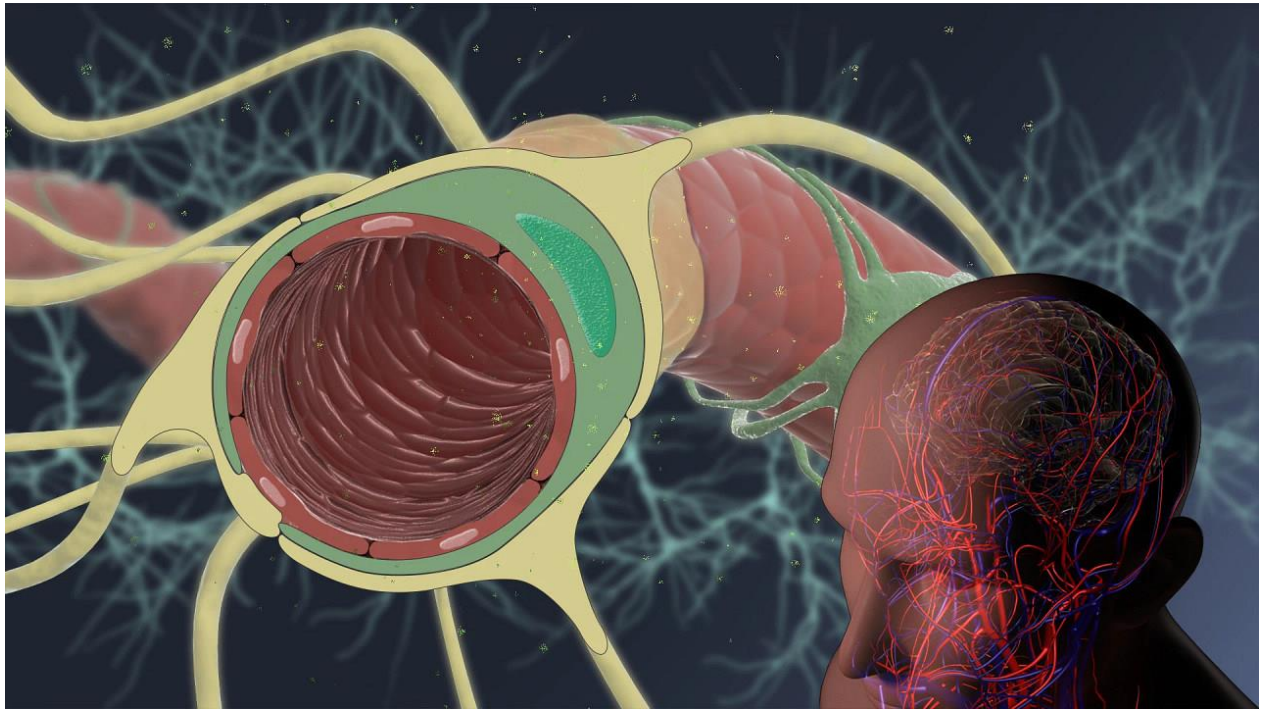
Organs-on-a-chip for different proposes



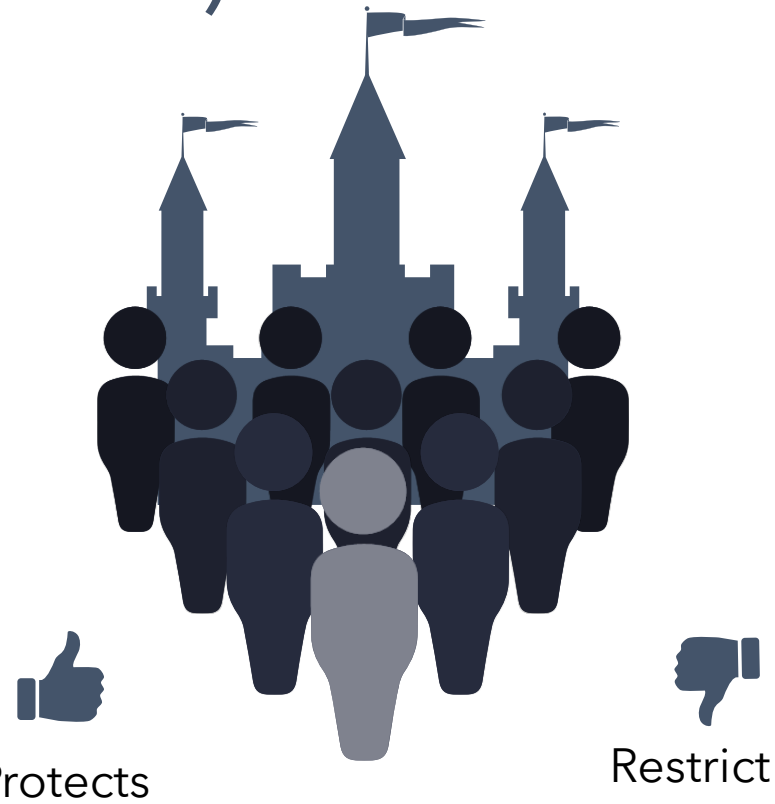


Conceptual schematic of a human-on-a-chip, a whole-body biomimetic device. Image: MIT

The Blood-Brain Barrier (BBB)



The human brain contains about 100 billion capillaries stretching about 650 kilometres (400 miles).



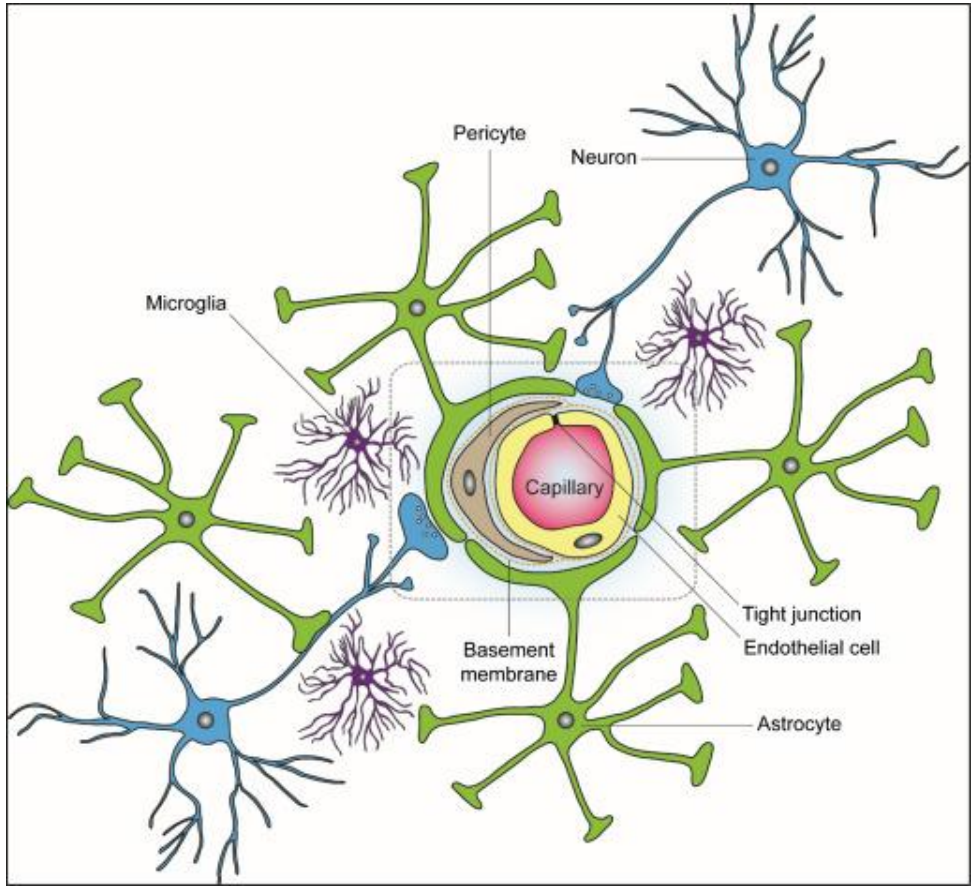
Protects

Allows only what is important for the CNS to pass through, protects from toxic or harmful agents .

Restrict

Limits the access of drugs that need to pass through the BBB to treat the CNS.

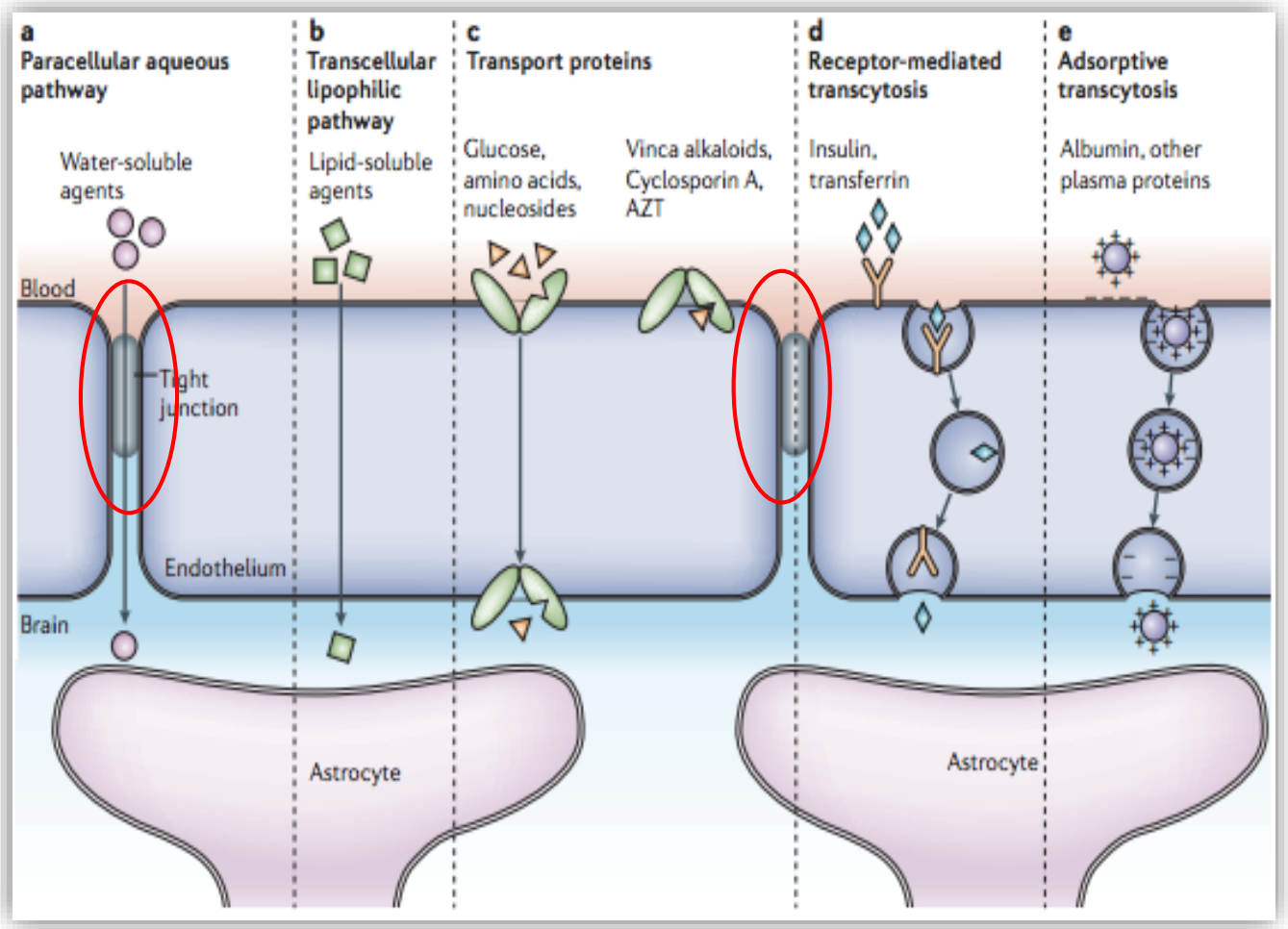
BBB composition



- Capilary lumen
- Endothelial cell
- Tigh junction
- Pericyte
- Basement membrane
- Astrocyte
- Neuron
- Microglia

Chin, E. & Goh, E. *Blood–brain barrier on a chip*. *Methods in Cell Biology* vol. 146 (Elsevier Inc., 2018).

BBB transport pathways



Abbott et al., Nature Reviews Neuroscience, 2006

Challenges in BBB studies

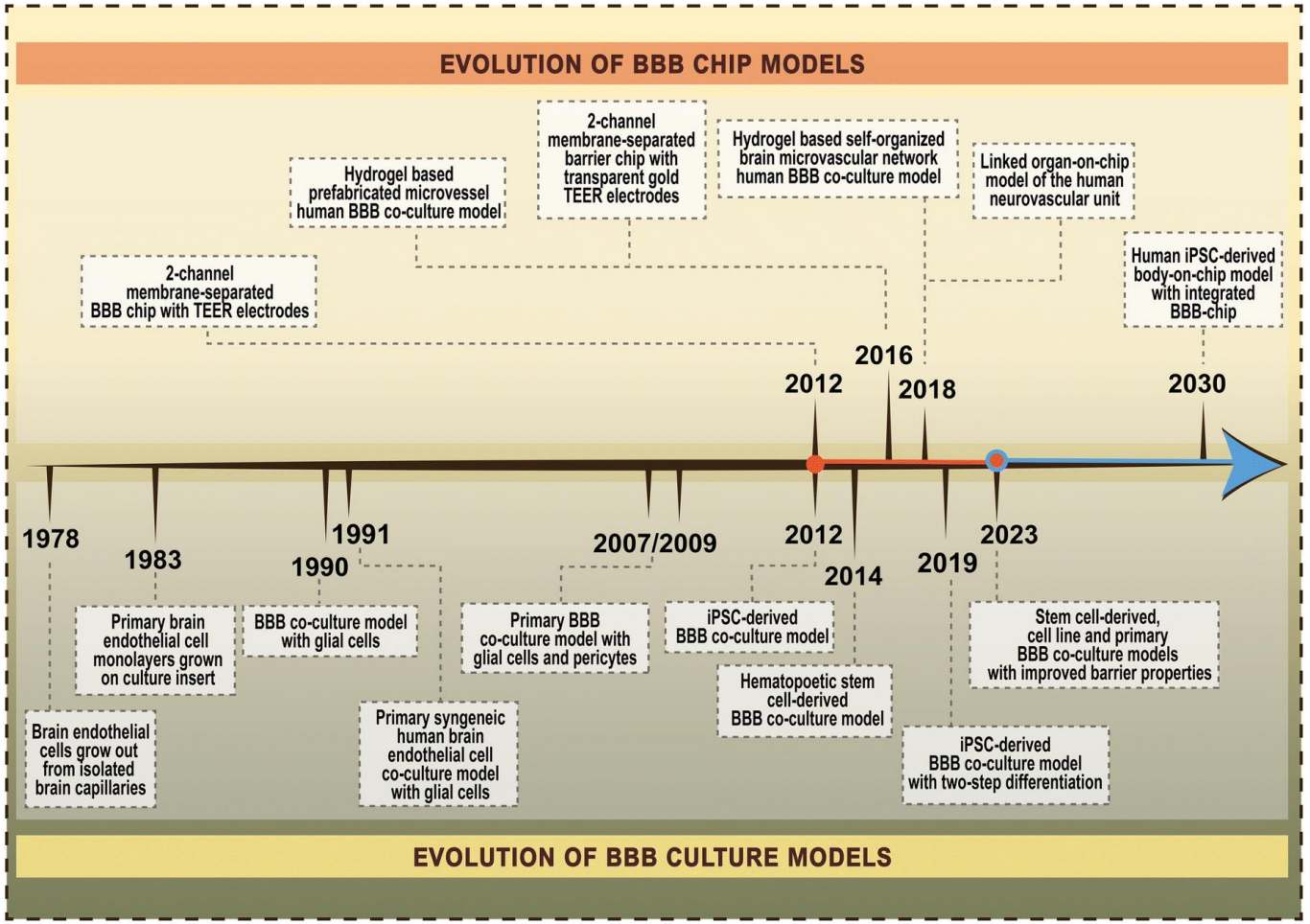
< 1% of small molecule across BBB

< 0,1% of big molecule across BBB

TEER: Transendothelial Electrical Resistance

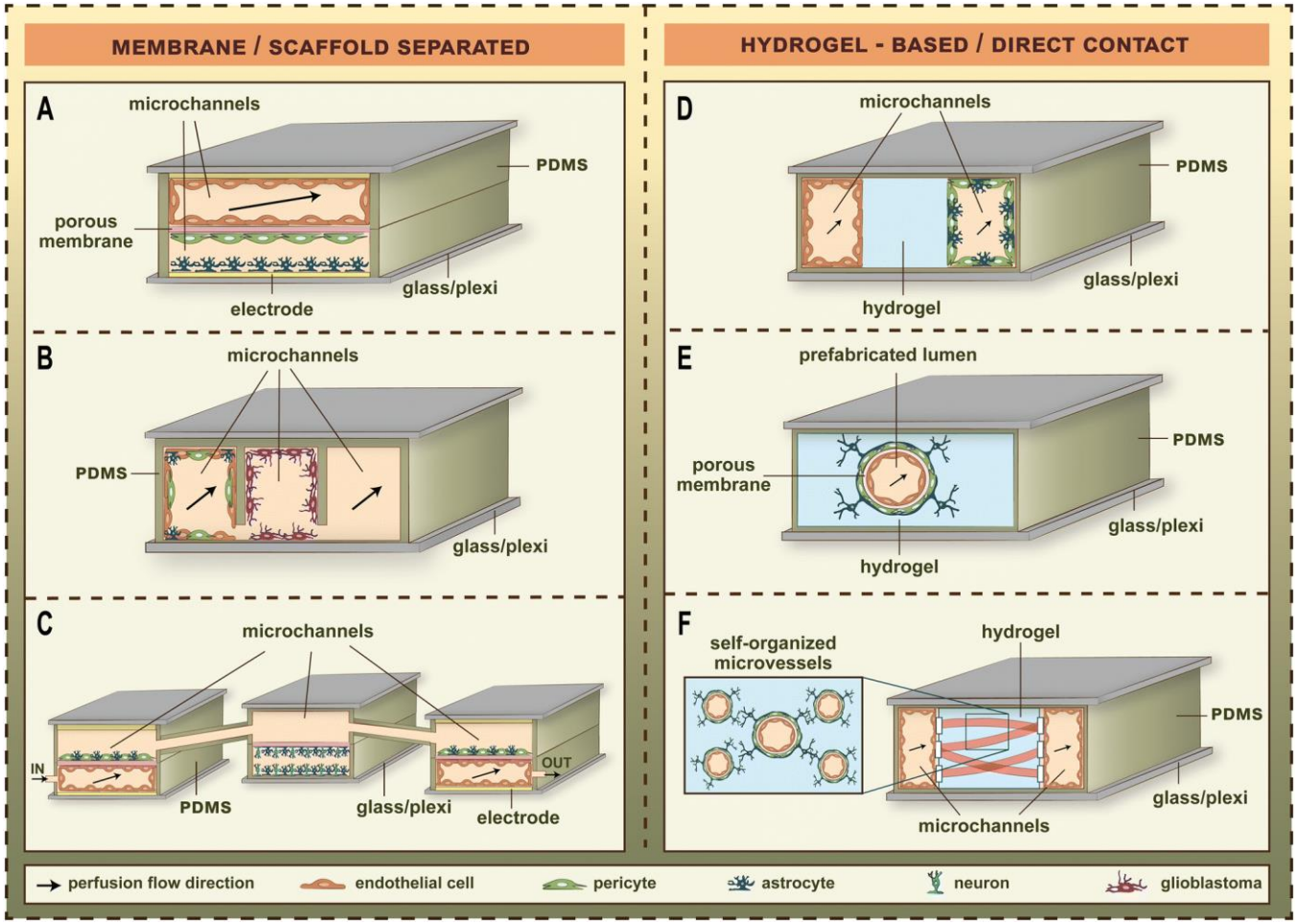
Abbott et al., Nature Reviews Neuroscience, 2006

Time-line of BBB models



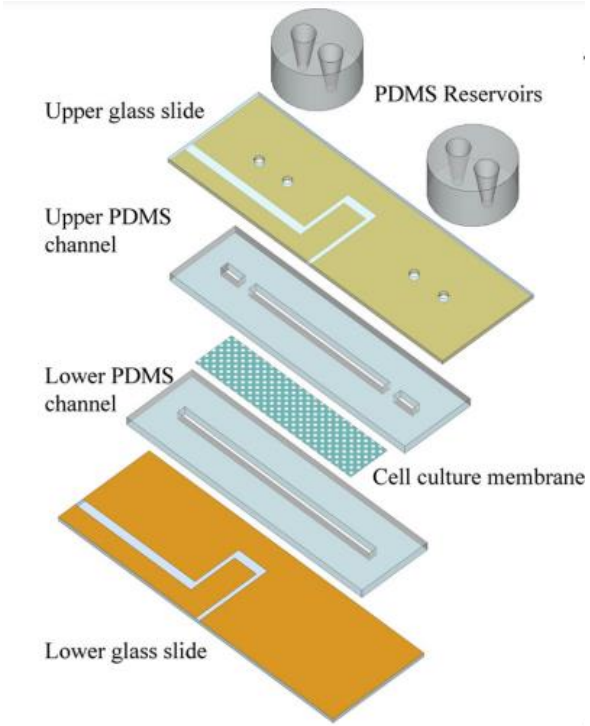
Deli, M. A. *et al.* Lab-on-a-chip models of the blood-brain barrier: evolution, problems, perspectives. *Lab Chip* **24**, 1030–1063 (2024).

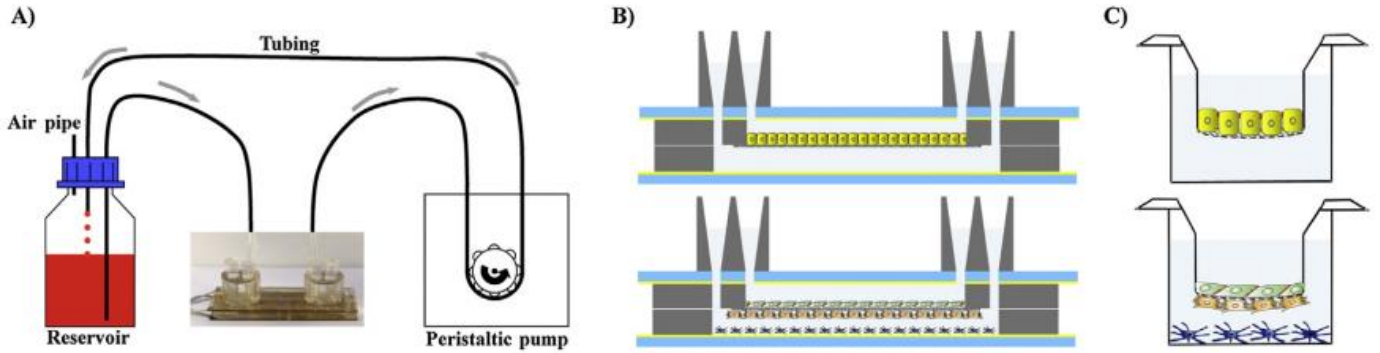
Types of BBB chip models



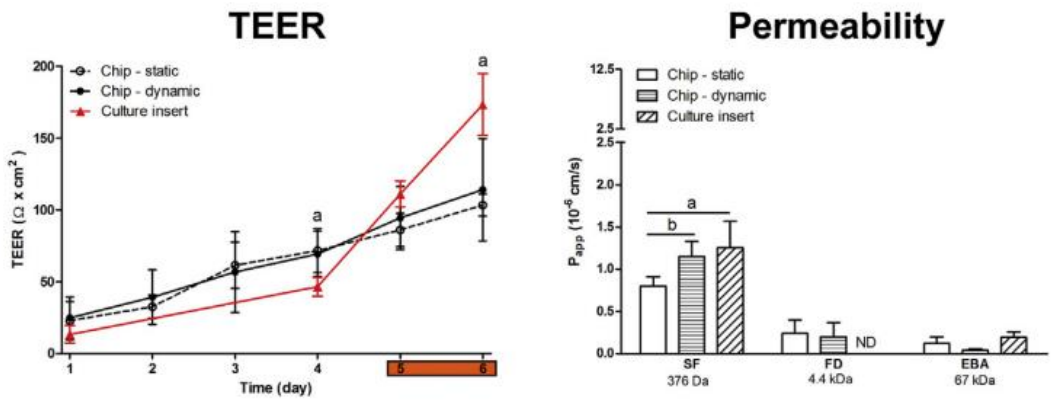
Deli, M. A. *et al.* Lab-on-a-chip models of the blood-brain barrier: evolution, problems, perspectives. *Lab Chip* **24**, 1030–1063 (2024).

Membrane separated



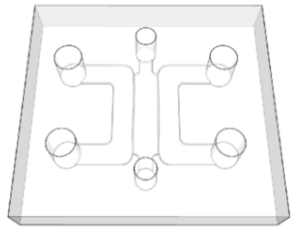


(A) Schematic drawing of the flow circuit. (B) Biochip setups with two types of cells. Epithelial cells (top, yellow cells) grow as monolayers on the porous membrane of the chip. Triple culture blood–brain barrier model using rat primary endothelial cells (green), pericytes (orange) and glial cells (blue) assembled in the biochip. **(C) Transwell cell culture inserts with the same arrangements for epithelial or endothelial cell models** as shown in the microchip



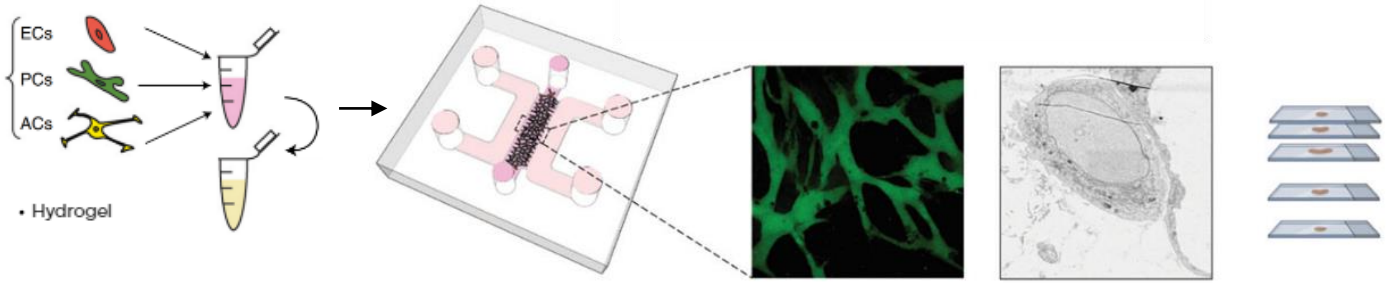
Walter, F. R. *et al.* A versatile lab-on-a-chip tool for modeling biological barriers. *Sensors Actuators, B Chem.* **222**, 1209–1219 (2016).

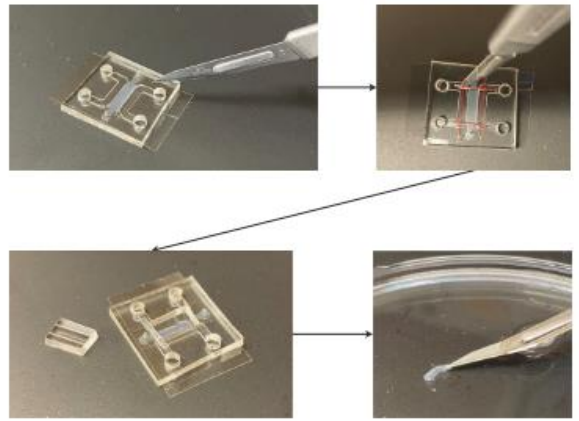
Hydrogel-based/ direct contact

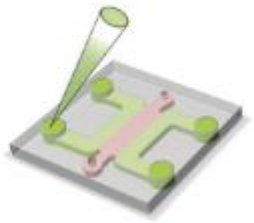


Macrodevice with
3D printed wafer

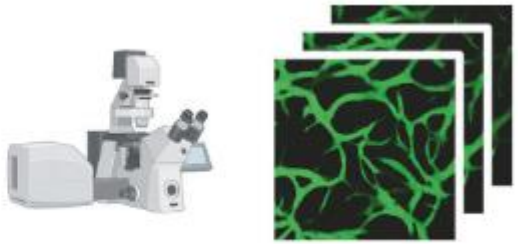
Device fabrication and
PDMS bonding (Steps 1–2)



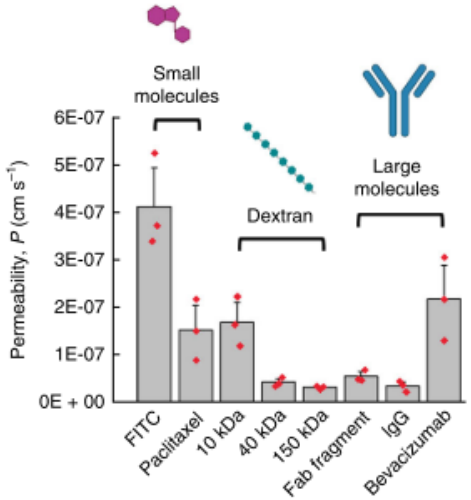




Device perfusion (Steps 21–24)



Confocal image acquisition (Steps 25–29)



Hajal, C. *et al. Engineered human blood–brain barrier microfluidic model for vascular permeability analyses*. *Nature Protocols* vol. 17 (Springer US, 2022).

TEER/impedance in BBB chips - challenges

Physiological TEER

no one value in vivo benchmark

direct measurements on TEER and shear stress are missing in the human brain vasculature

Measurement of TEER is influenced by:

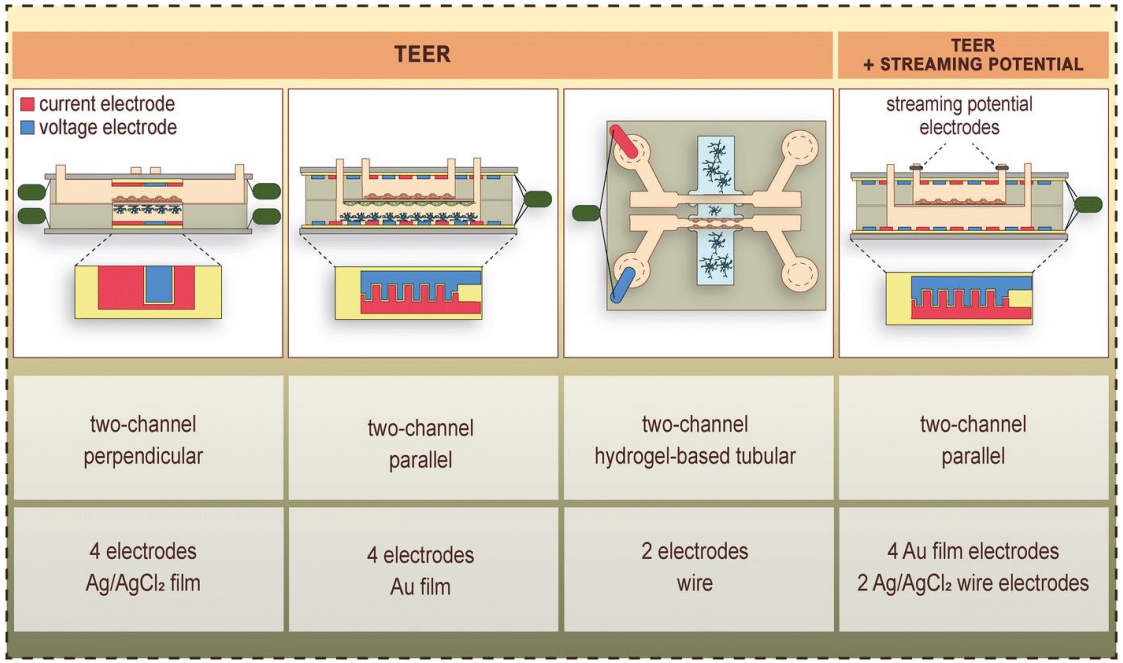
electrode type, number, material, positioning

temperature

viscosity

TEER values can differ by orders of magnitude

same BBB models in diferente studies



Deli, M. A. *et al.* Lab-on-a-chip models of the blood-brain barrier: evolution, problems, perspectives. *Lab Chip* **24**, 1030–1063 (2024).

BBB cells in chips - challenges

Immortalized brain endotelial cell lines

nonphysiological characteristics, weak barrier properties

Primary brain endotelial cells

species diferences, hard to source

Stem cell derived brain

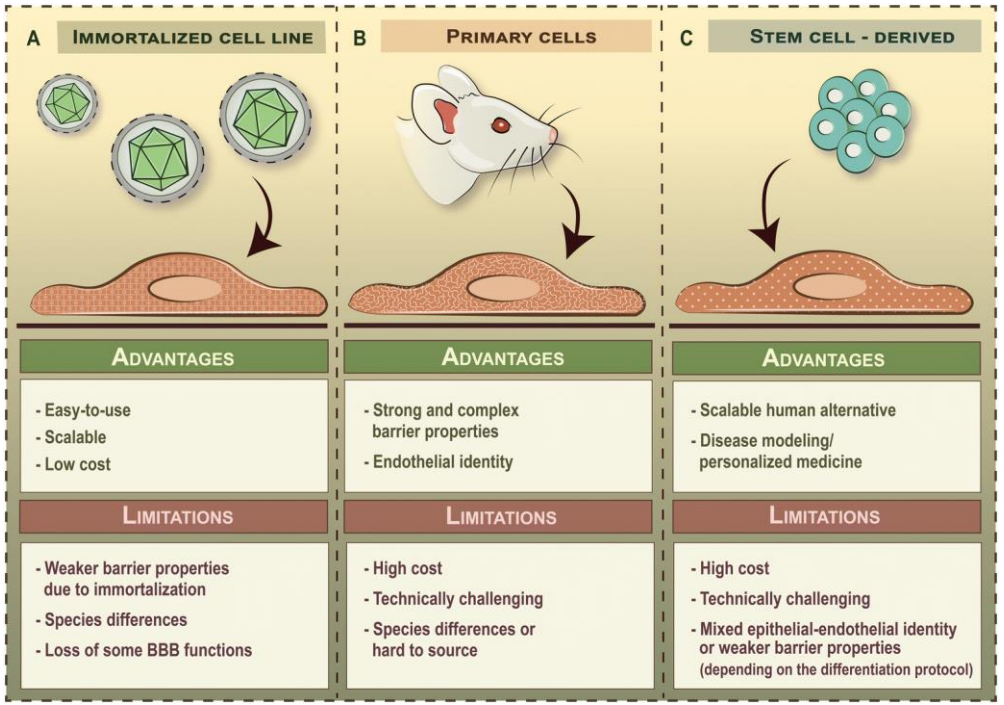
tight barriers and mixed neuroepithelial and endotelial identity

Enfothelial idenditty and low tighsness

Lack of guidelines

For barrier properties in BBB-on-a-chip models

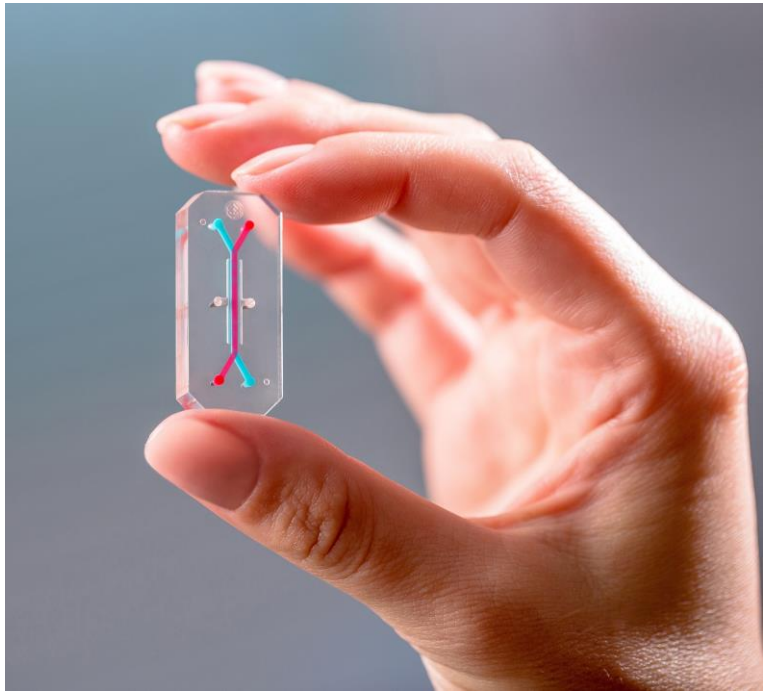
Hard to comparate and benchmark results from diferente laboratories



Deli, M. A. *et al.* Lab-on-a-chip models of the blood-brain barrier: evolution, problems, perspectives. *Lab Chip* **24**, 1030–1063 (2024).

Perspectives - Needed

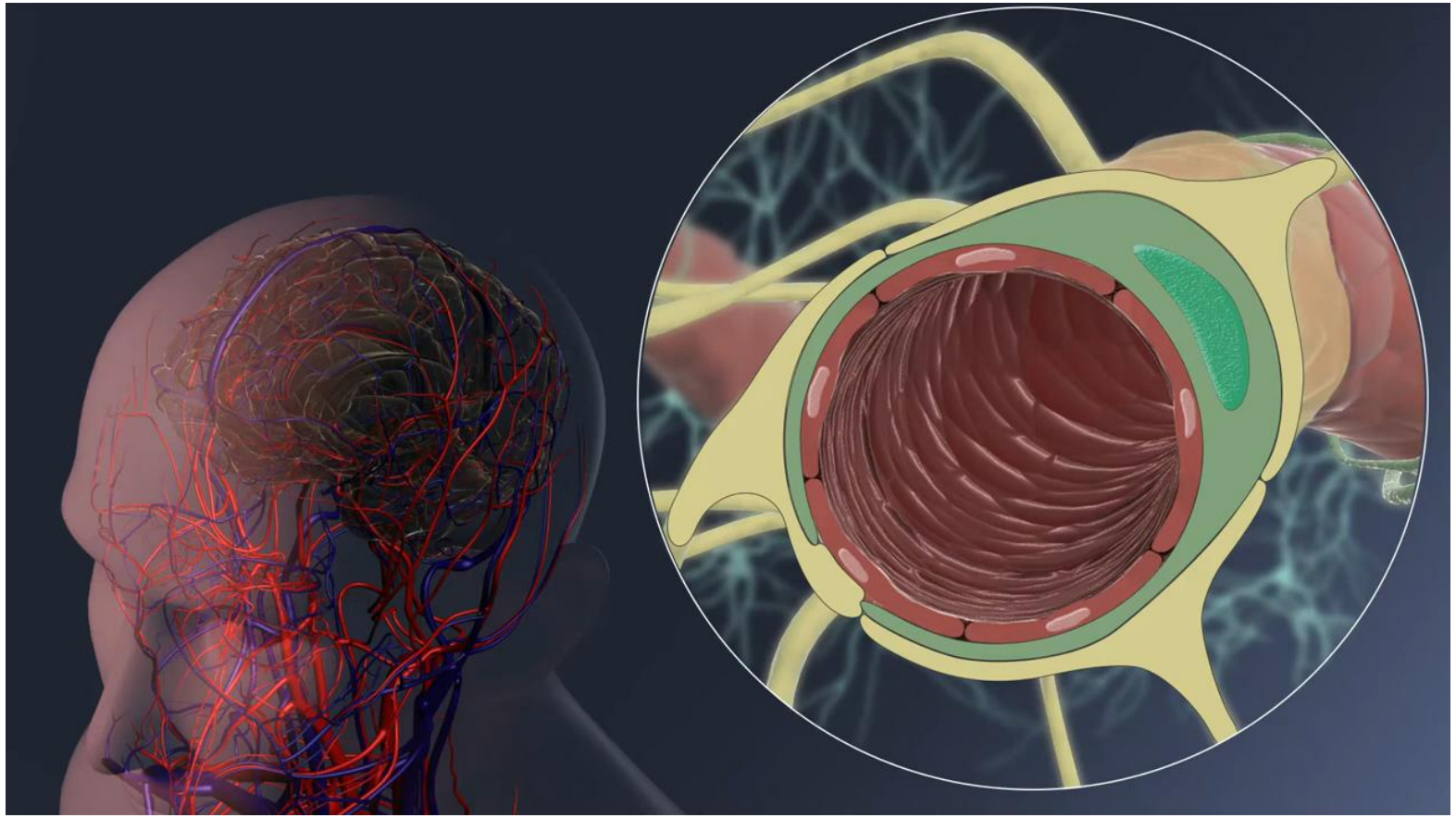
- Direct in vitro/in vivo measurements and comparisons
- Organ and zonation-specific effects of shear stress in the vasculature
- Expertise in materials Science, bioengineering, stem cell, vascular BBB biology
- Better integration of these diverse disciplines
- Consensus paper with guidelines



Deli, M. A. *et al.* Lab-on-a-chip models of the blood-brain barrier: evolution, problems, perspectives. *Lab Chip* **24**, 1030–1063 (2024).

“Brain Targeting Program”

- Strategies to get drugs to the brain more effectively.



Wyss Institute for Biologically Inspired Engineering. *Brain Targeting Program* https://wyss.harvard.edu/collaboration/brain-targeting-program/.

Thank you!

sheilagomes@usp.br