Biomechanics in Organ-on-Chip Systems

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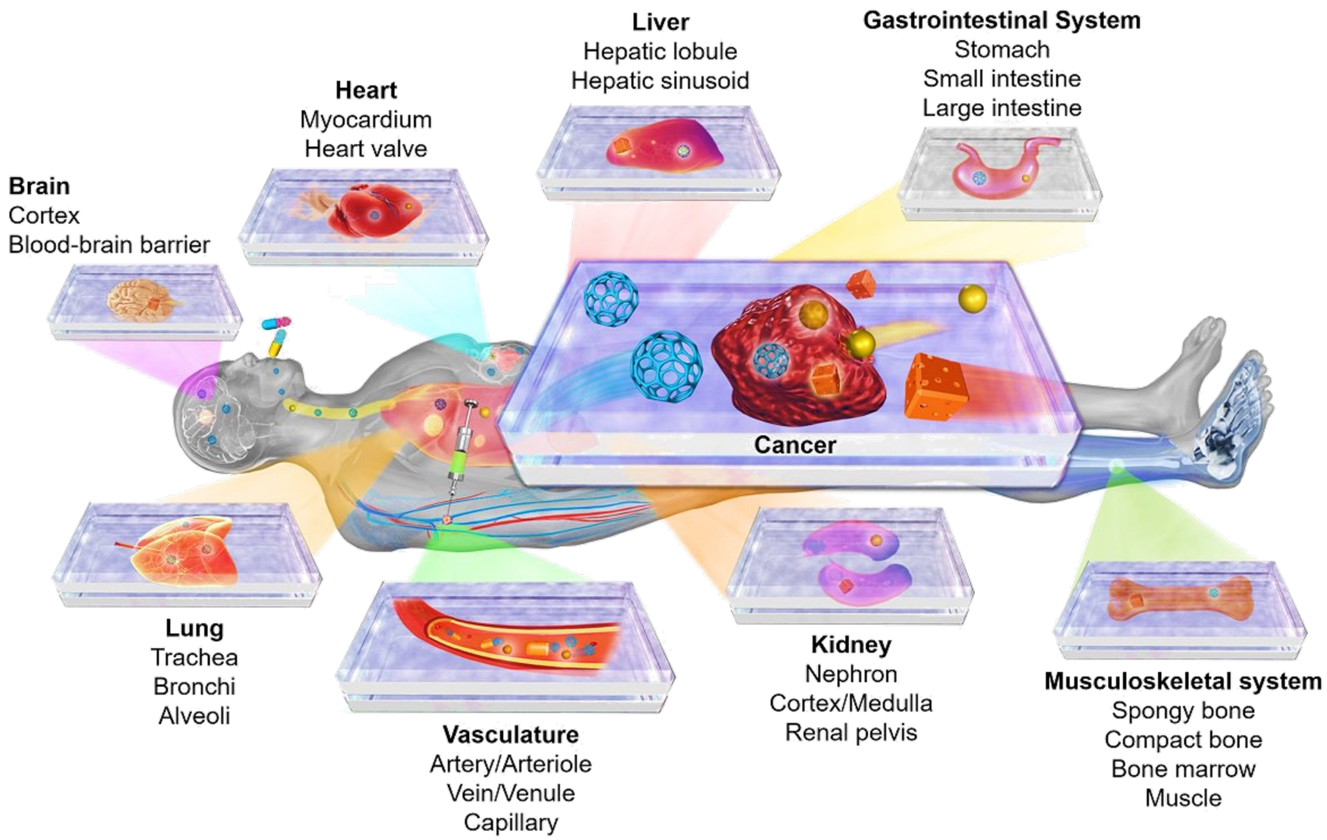
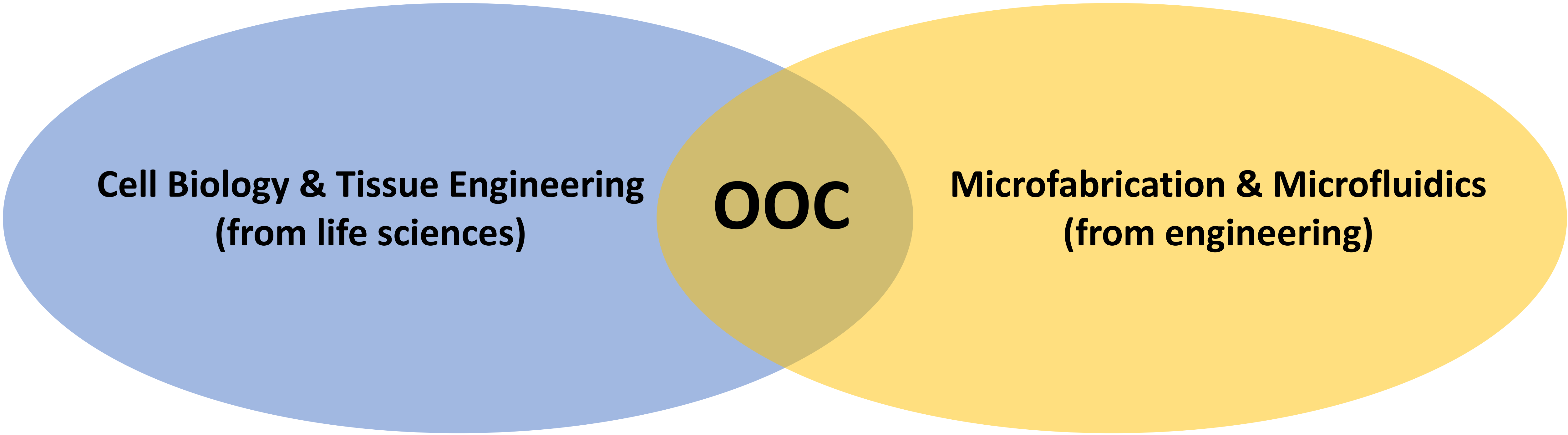
What are Organ-on-chips (OOC)?



Zhang YS et al. (*Drug Discovery Today*, 2017)

- Microfluidic devices mimicking organs
- Realistic/functional tissue microenvironments

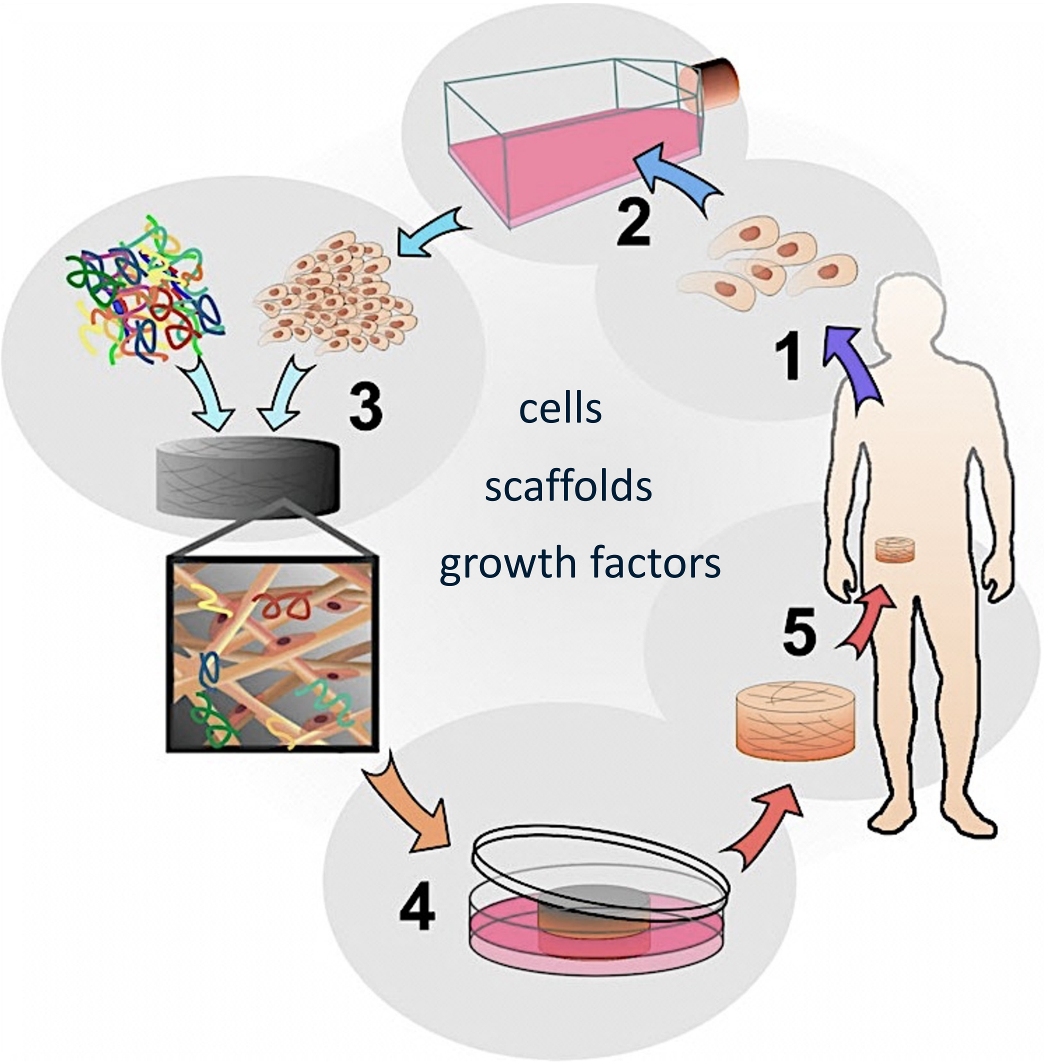
The OOC is a multidisciplinary field



The OOC is a multidisciplinary field

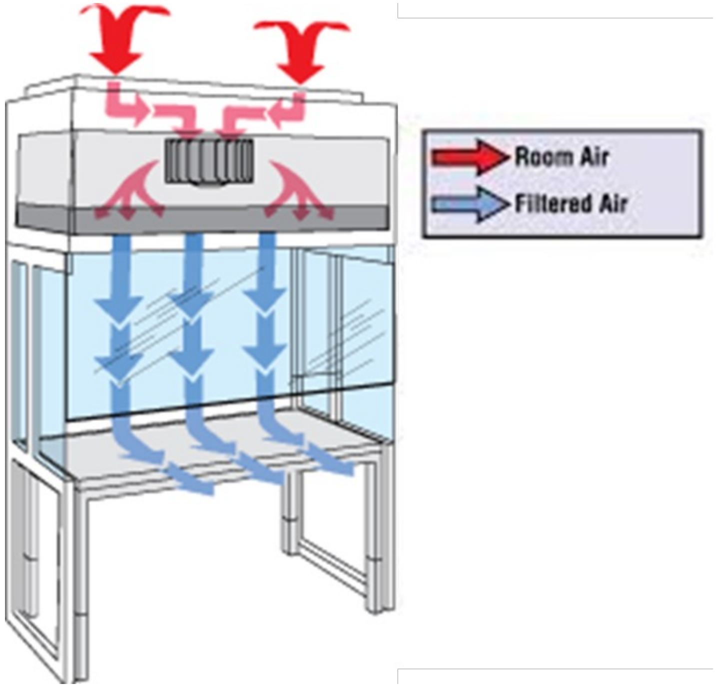
Cell Biology & Tissue Engineering
(from life sciences)

Tissue engineering concepts



The goal is to restore, maintain, or improve damaged tissue or organs

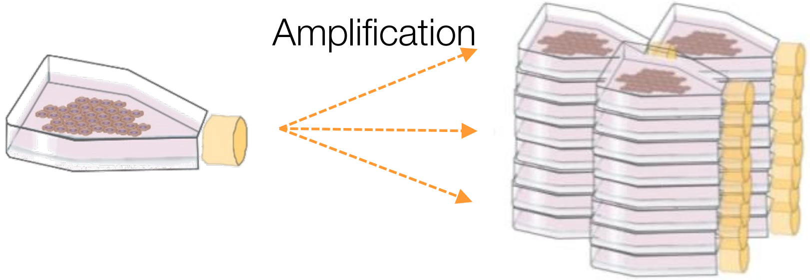
Basics of mammalian cells culture

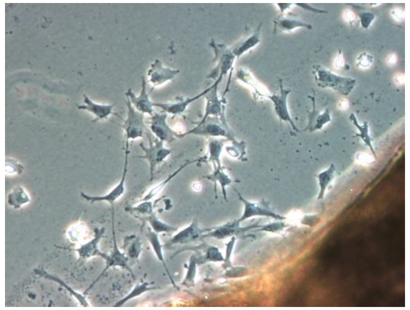


For cell growth

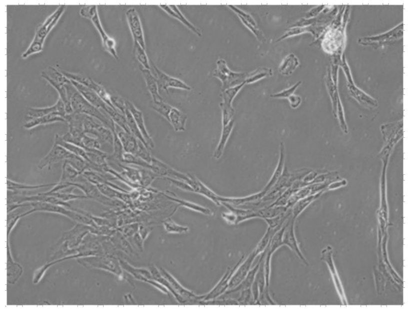
For cell experiments

For cell primary explant

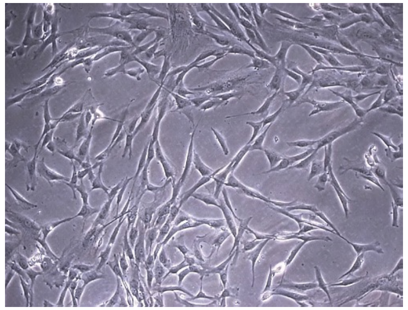




Bone

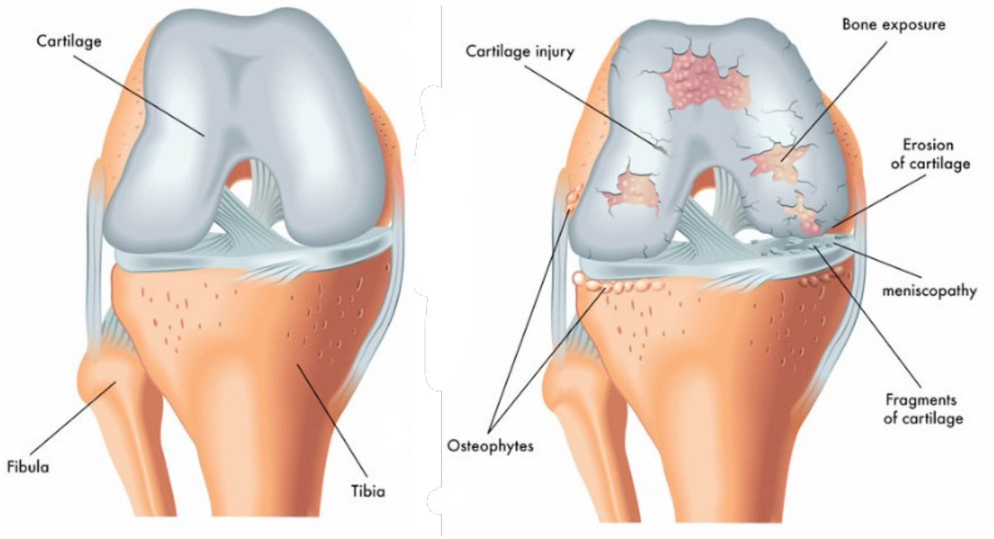


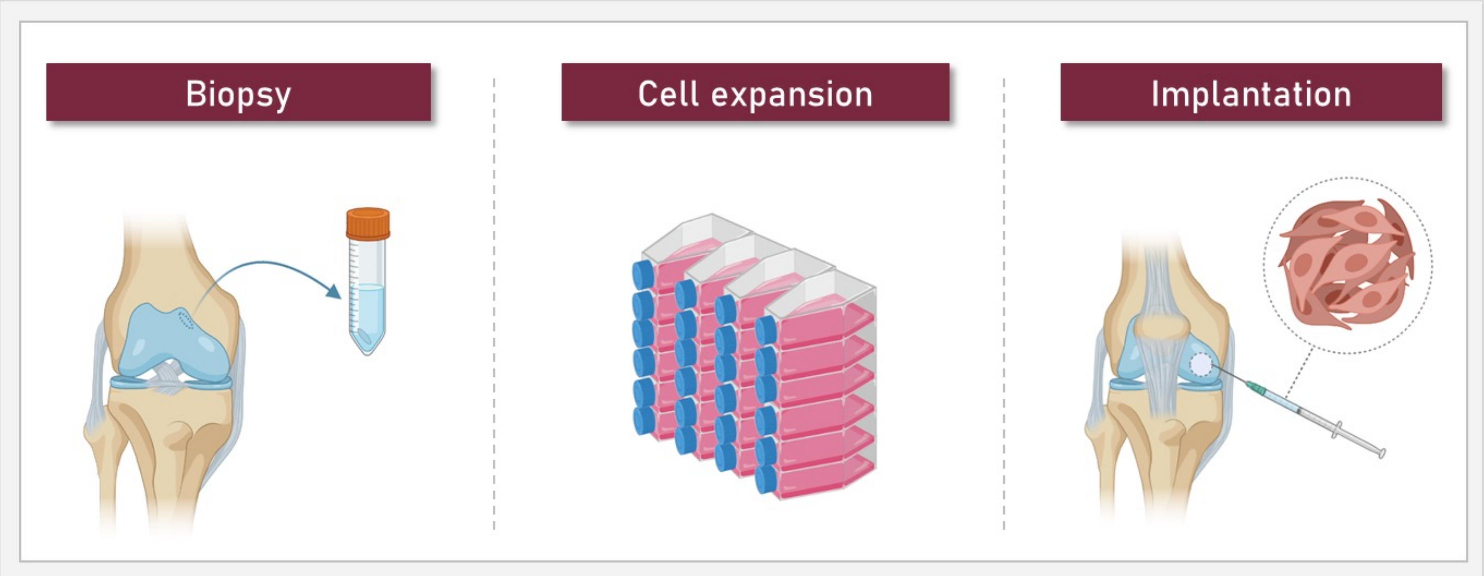
Cartilage



Fibroblasts

Example of Tissue Engineering: Autologous Chondrocytes Implantation (ACI)





Brittberg et al. (*N Engl J Med*, 1994)

The OOC field originates from the intersection of multiple disciplines

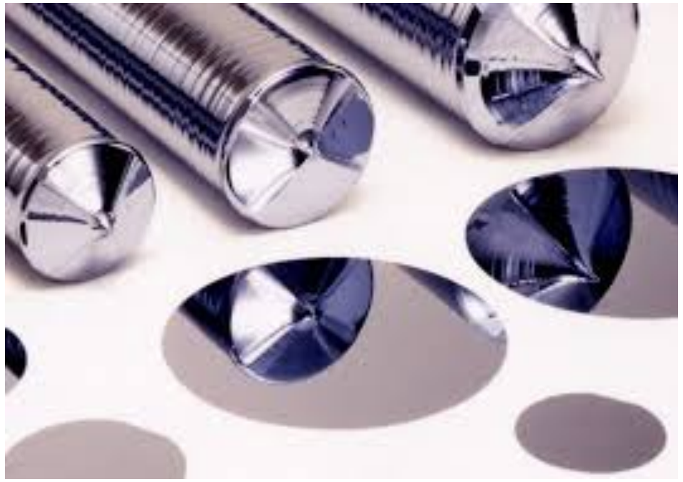
Cell Biology & Tissue Engineering (from life sciences)

The OOC field originates from the intersection of multiple disciplines

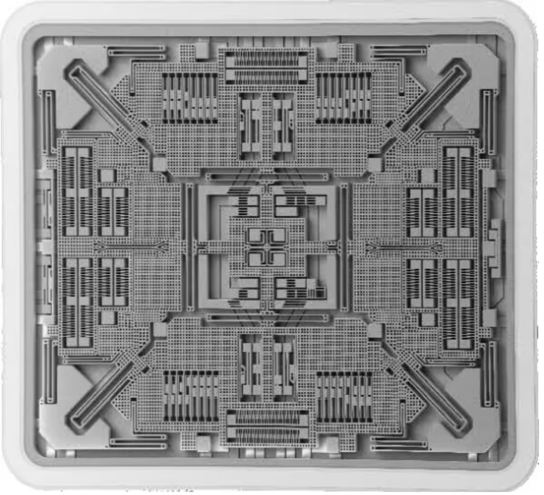
Microfabrication & Microfluidics (from engineering)

Lab-on-chips development

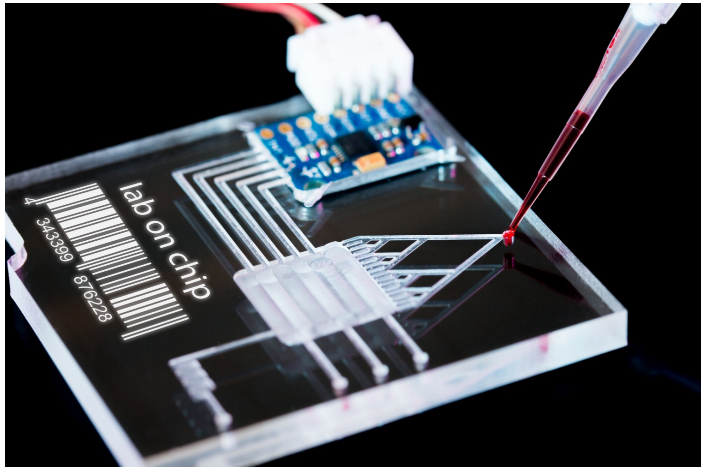
- LOC technology derived from the industry of microelectronics:
  - Use of **microelectronics materials** for which fabrication techniques are available and mature (1980s–2000s)
  - Provided the tools to miniaturize and precisely control fluidic environments at the micrometer scale



Silicon-based 3-12” wafers

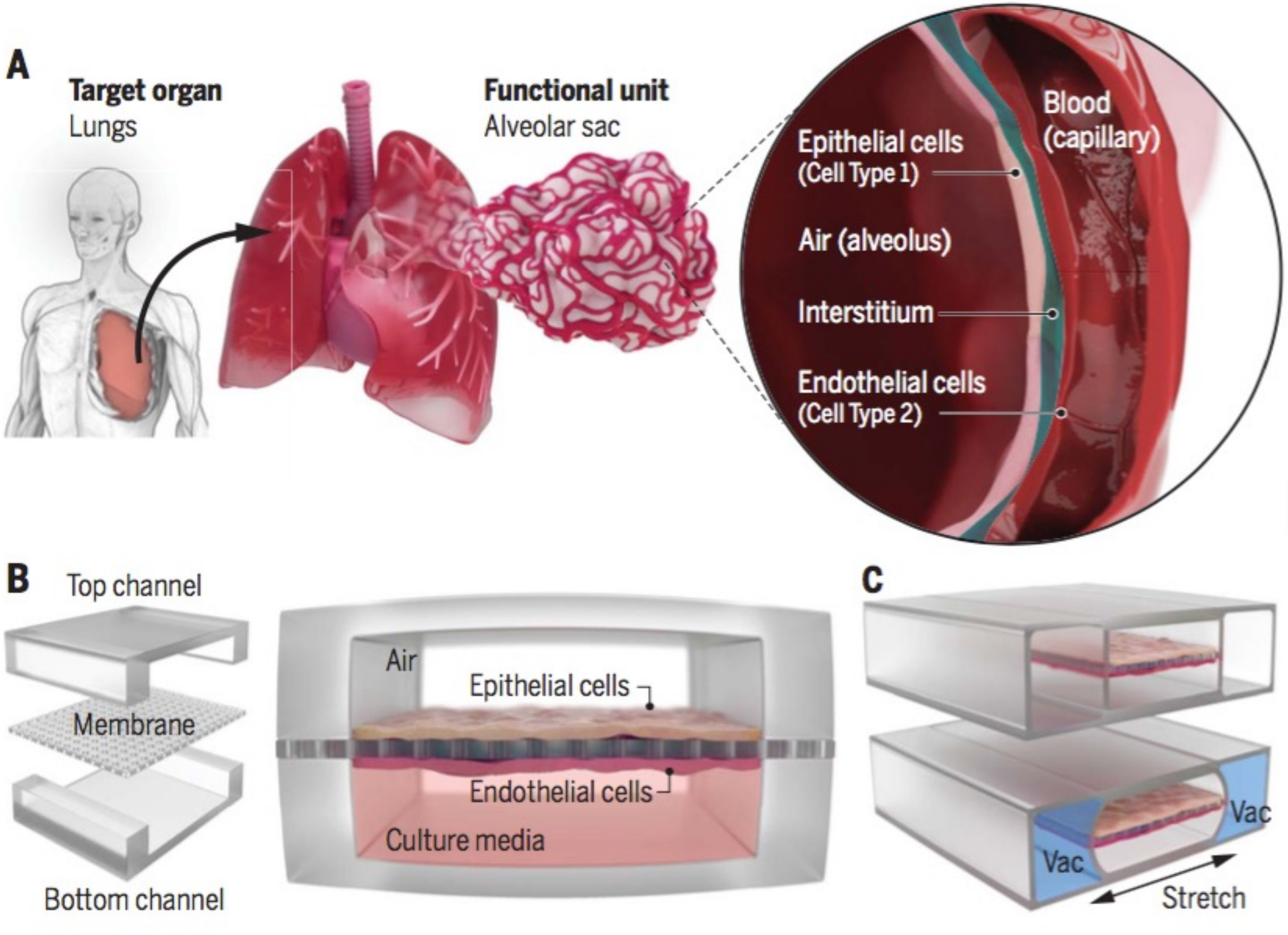


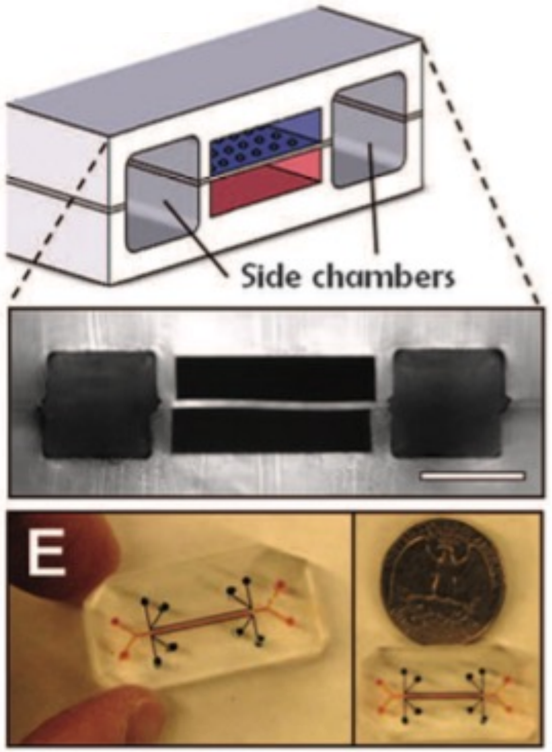
MEMS



Microfluidic systems

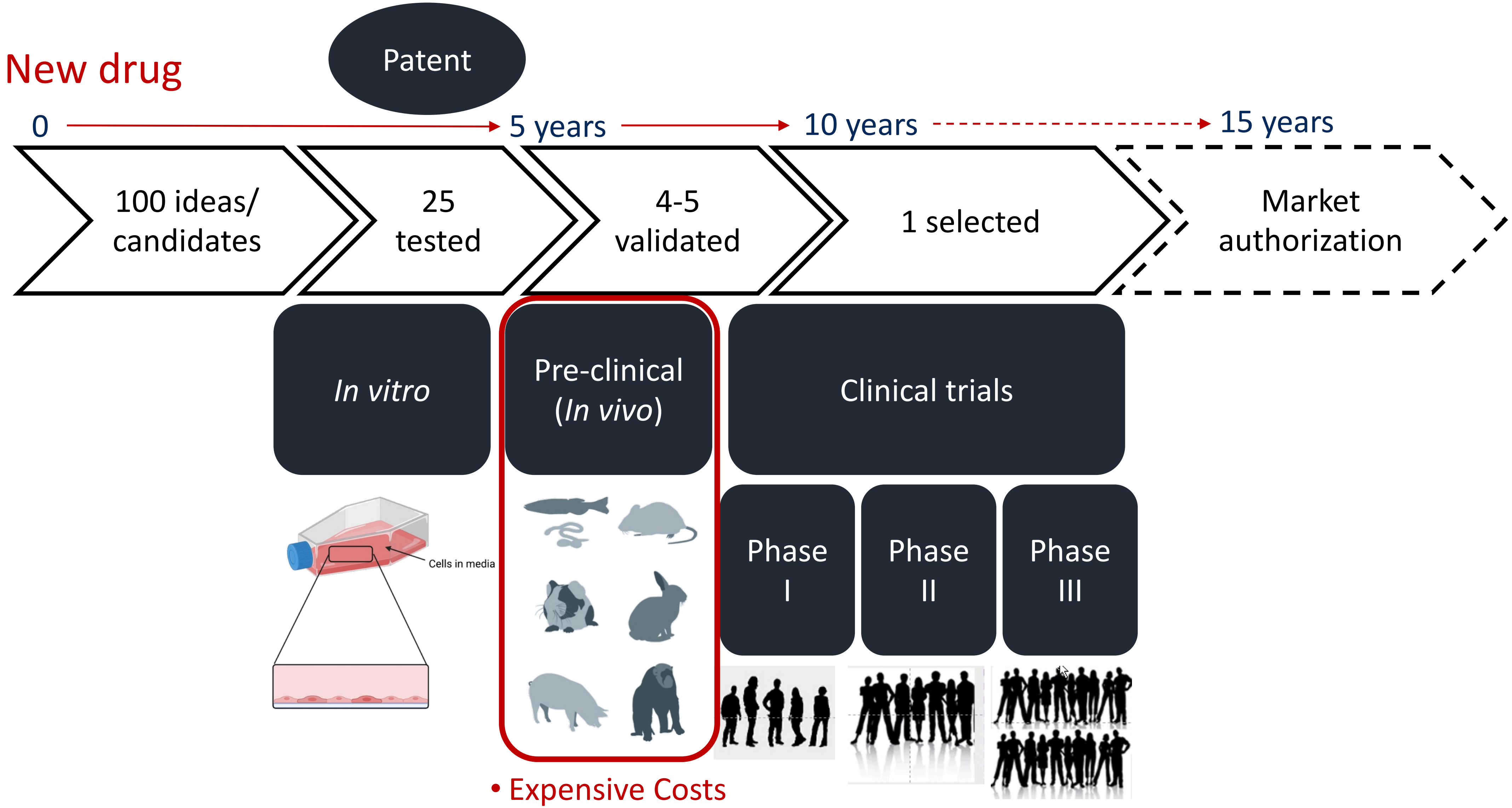
Why to develop these OOC systems?





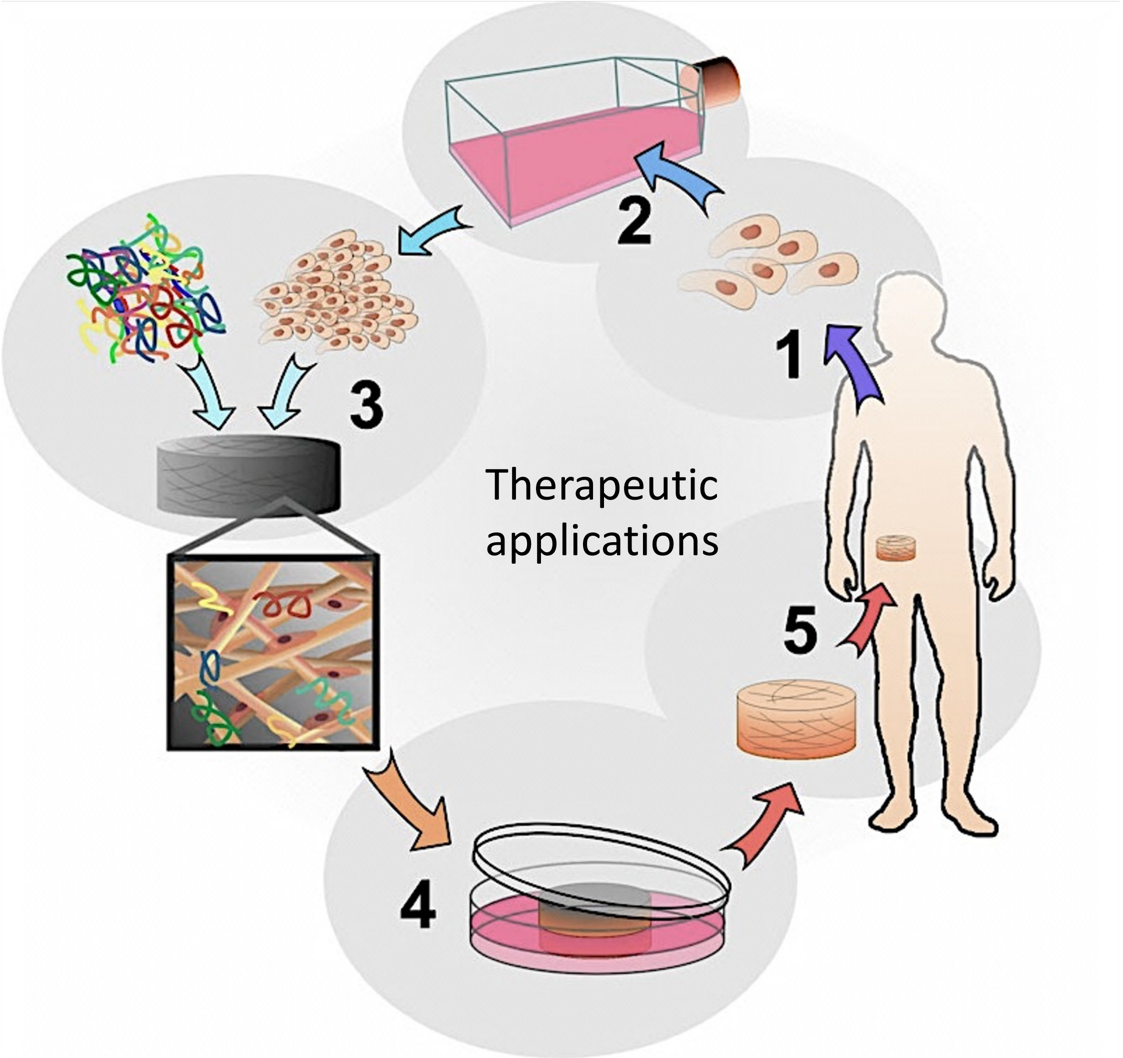
Park SE et al. (*Science,* 2019)
Dongeun H et al. (Sciences, 2010)

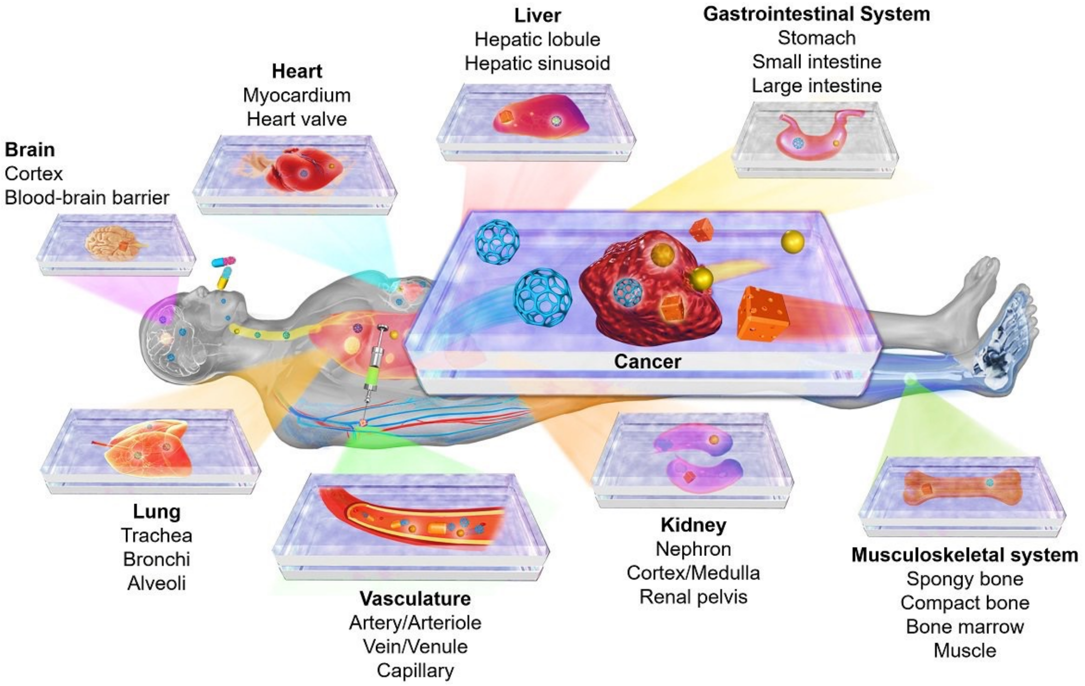
The pathway of a therapeutic product



- Expensive Costs
- Ehical concerns

TE shifting paradigm to organoid models

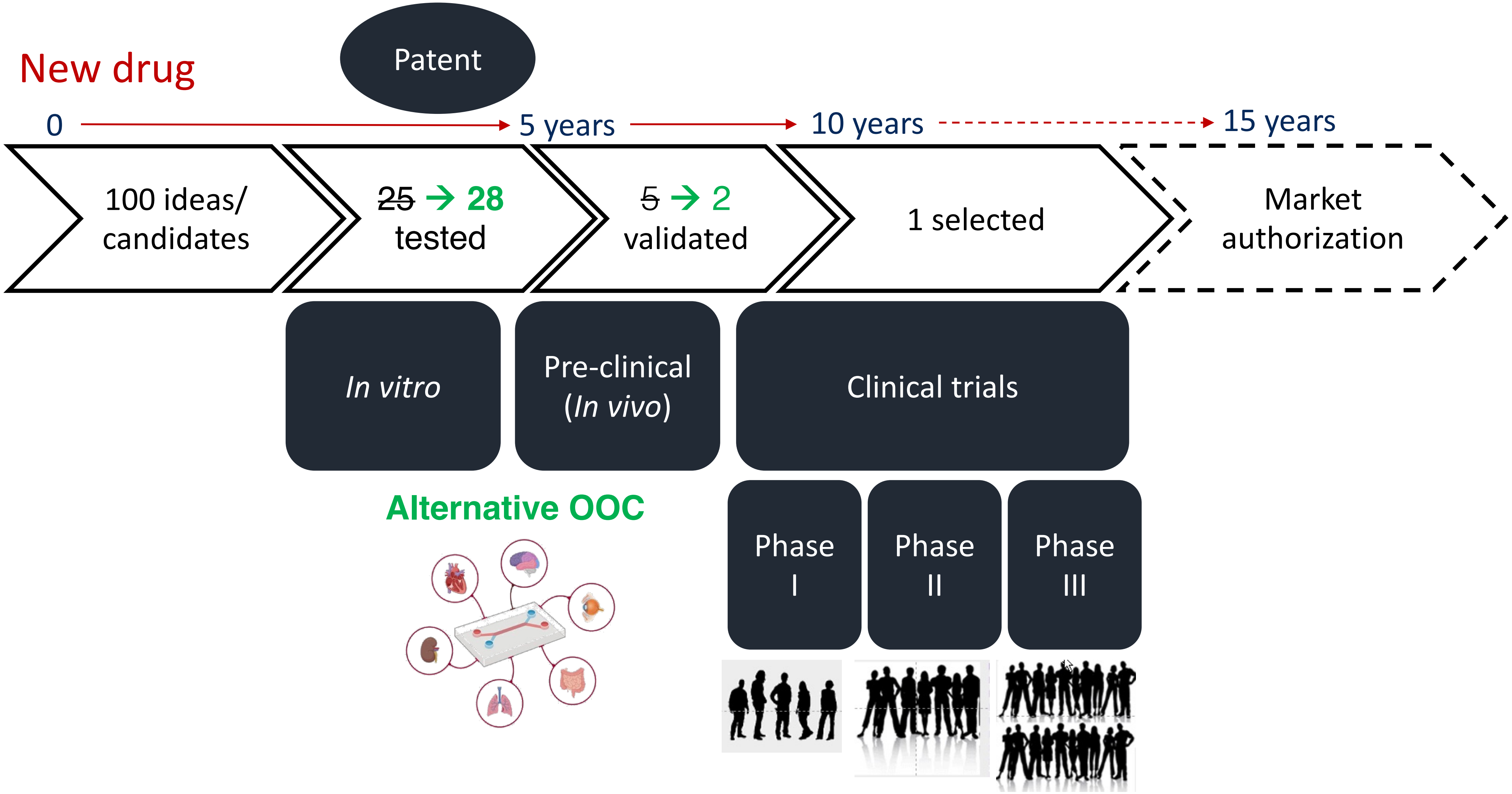




- Disease modeling (CRISPR gene editing)
- Personalized medicine (e.g. drug screening)

Zhang YS et al. (*Drug Discovery Today*, 2017)

Alternative to animal experimentation (3R)



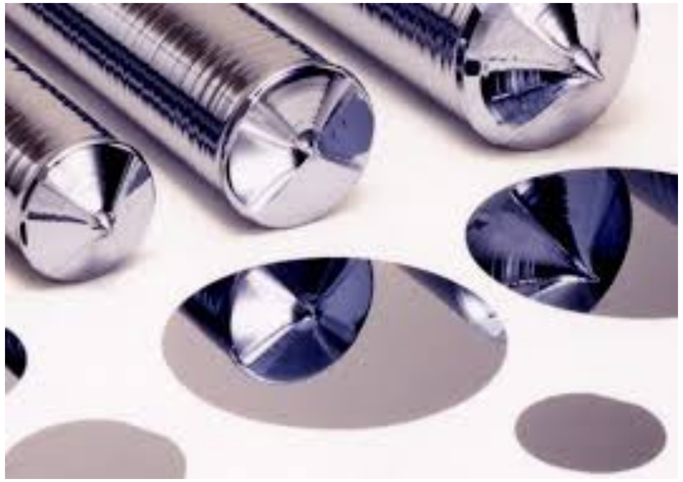
Main advantages of organ-on-chip systems

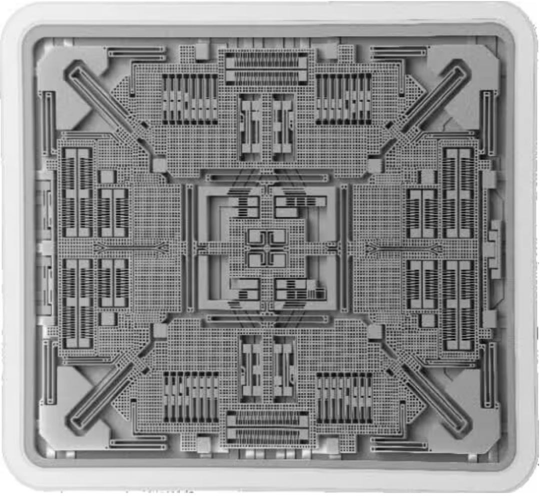
- Control over **spatiotemporal organization** of *in vivo*-like tissue architectures
- Ability to precisely control the **amount**, **duration** and **intensity** of the biomechanical or biochemical **cues**
- Useful for initial screening (**3R**s principle)
- Capability of **monitoring in real time** the effects of applied mechanical forces on cell, tissue and organ functions.

Fabrication methods

Lab-on-chips development

- LOC technology derived from the industry of microelectronics:
  - Use of **microelectronics materials** for which fabrication techniques were available and mature
  - Well-known physical and chemical **properties** and well-characterized **surface derivatization chemistries**



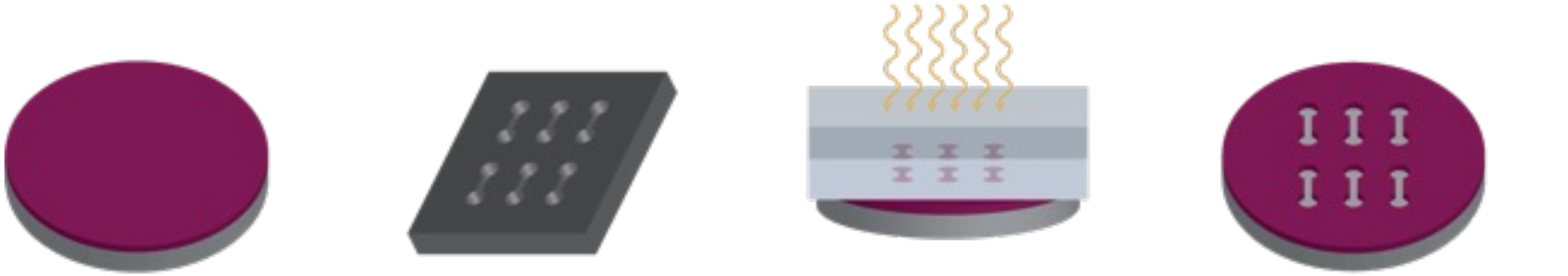


Silicon-based 3-12” wafers

Clean room

1. Photolithography

Photolithography Process



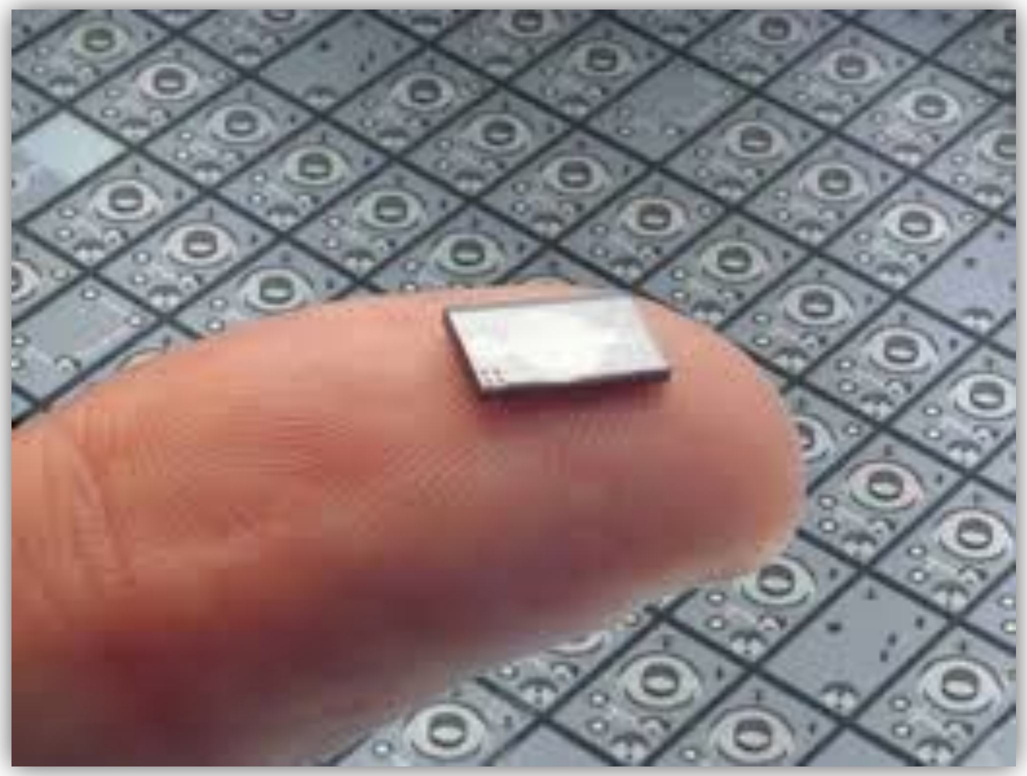
Silicon wafer
We begin with a clean silicon wafer spin coated with photoresist

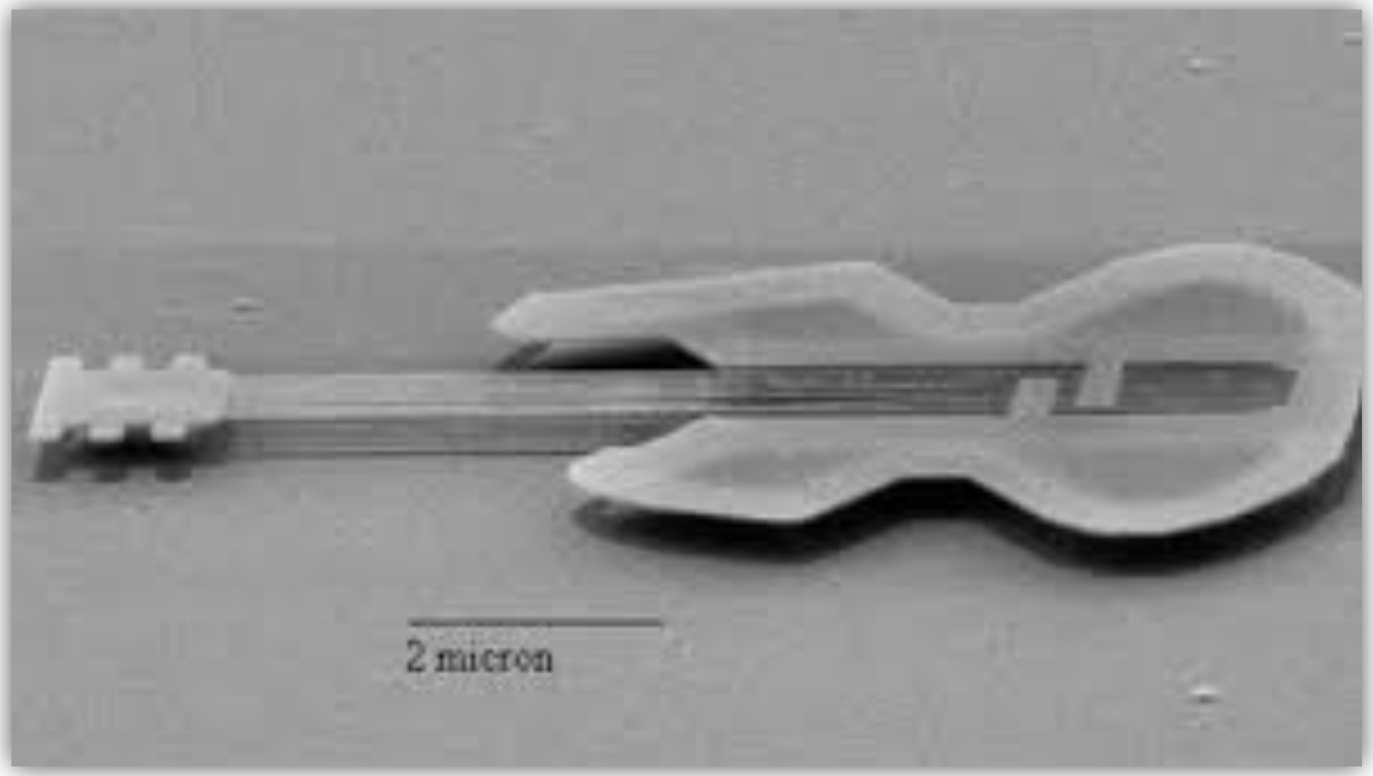
Photomask
A glass or mylar mask coated with an opaque film defines the features

Exposure
A mask aligner is used to pass UV light through the mask onto the wafer

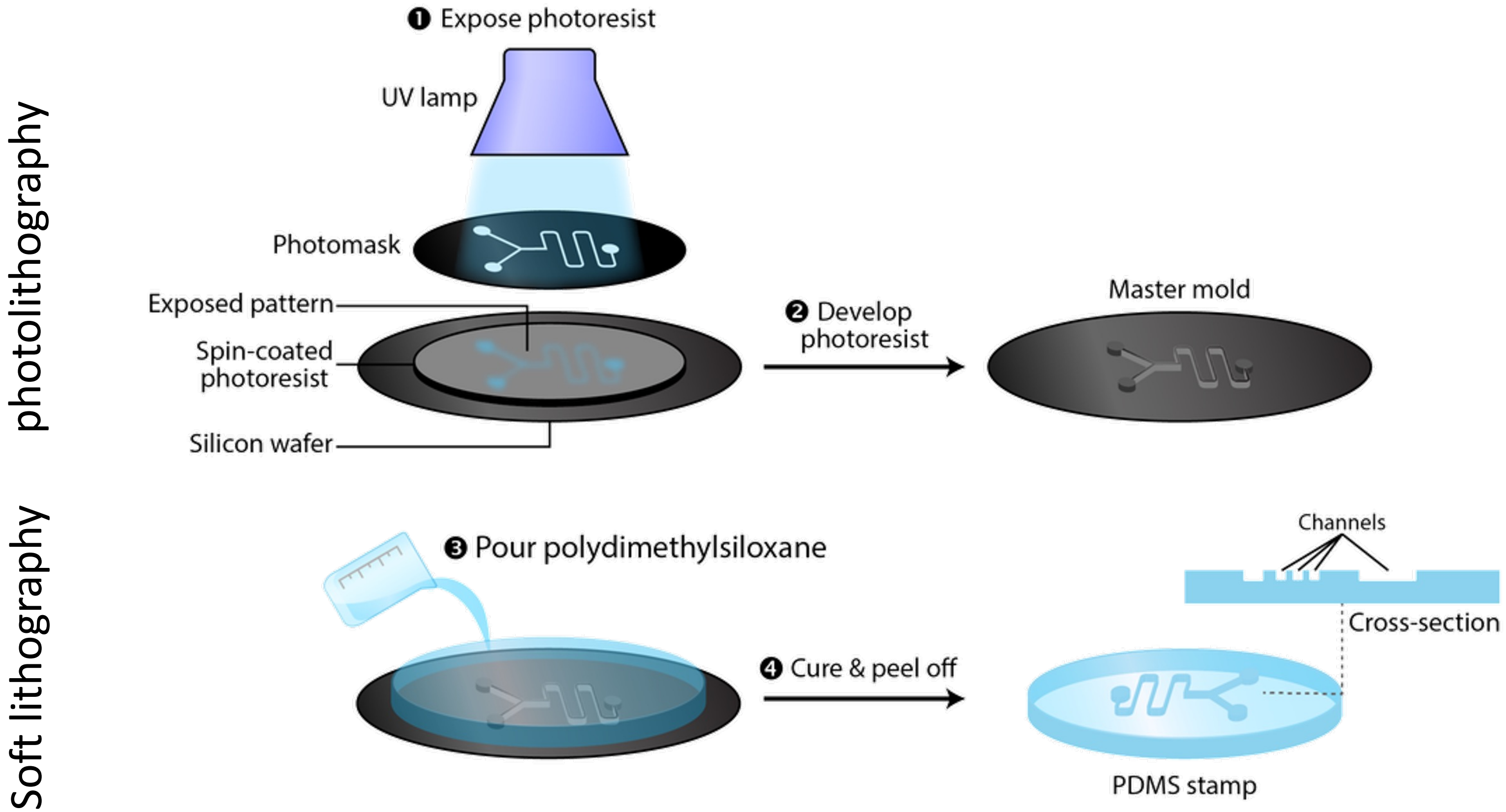
Development
Exposed resist is washed away while unexposed resist remains

Example

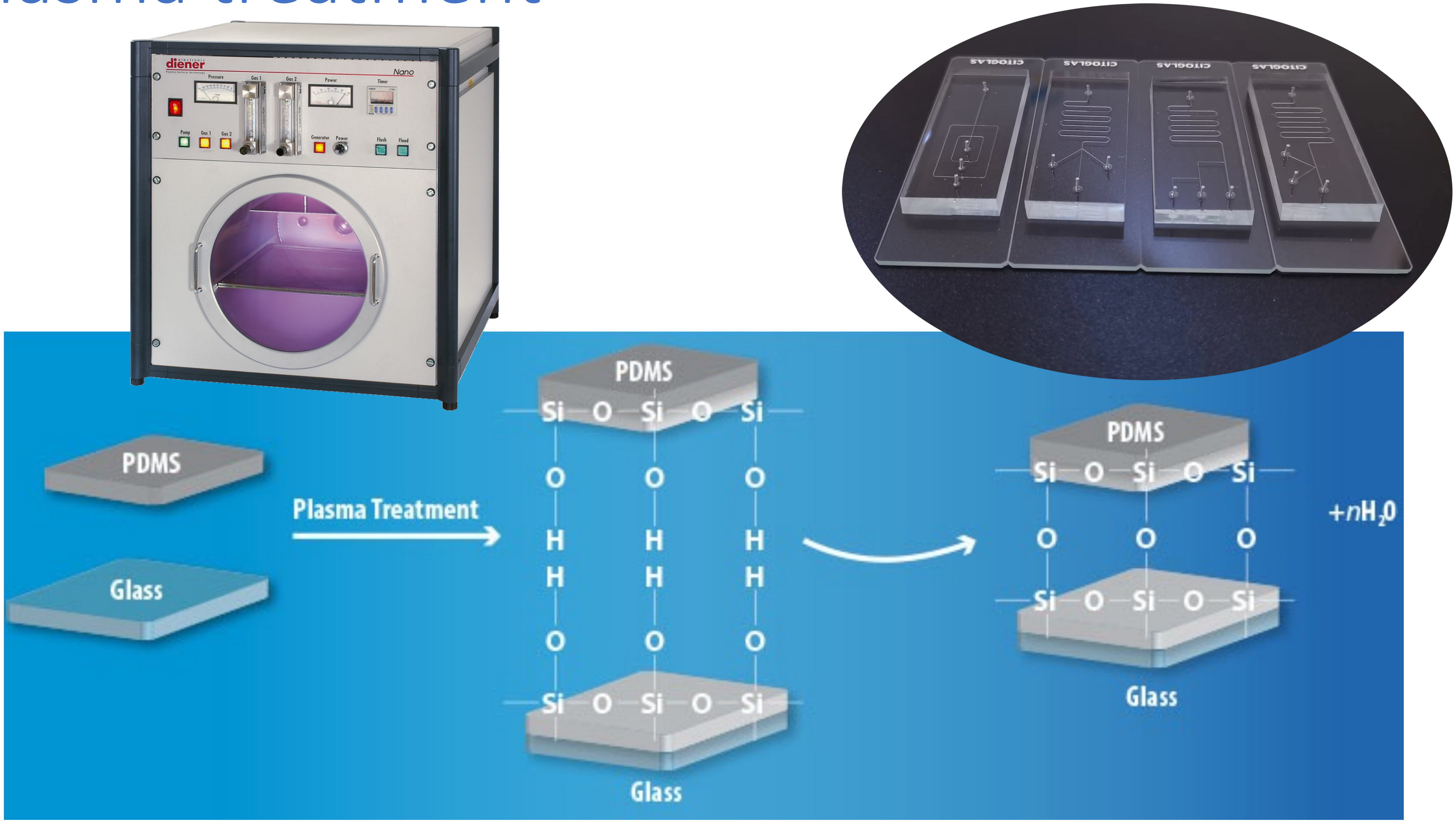




2. Soft lithography (Polymer)



Plasma treatment

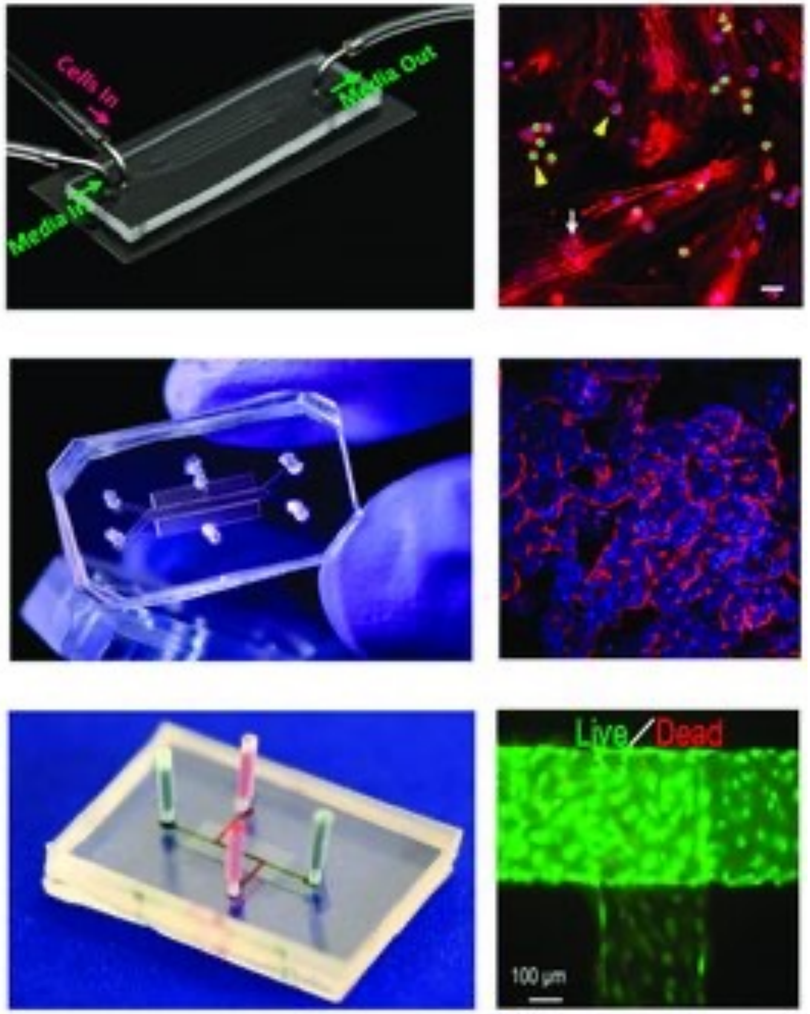


https://plasmatreatment.co.uk/knowledge-base/knowledge-articles/101-plasma-treatment-of-pdms-leads-to-improved-adhesion-bonding

PDMS: a key polymer for applications in Bio

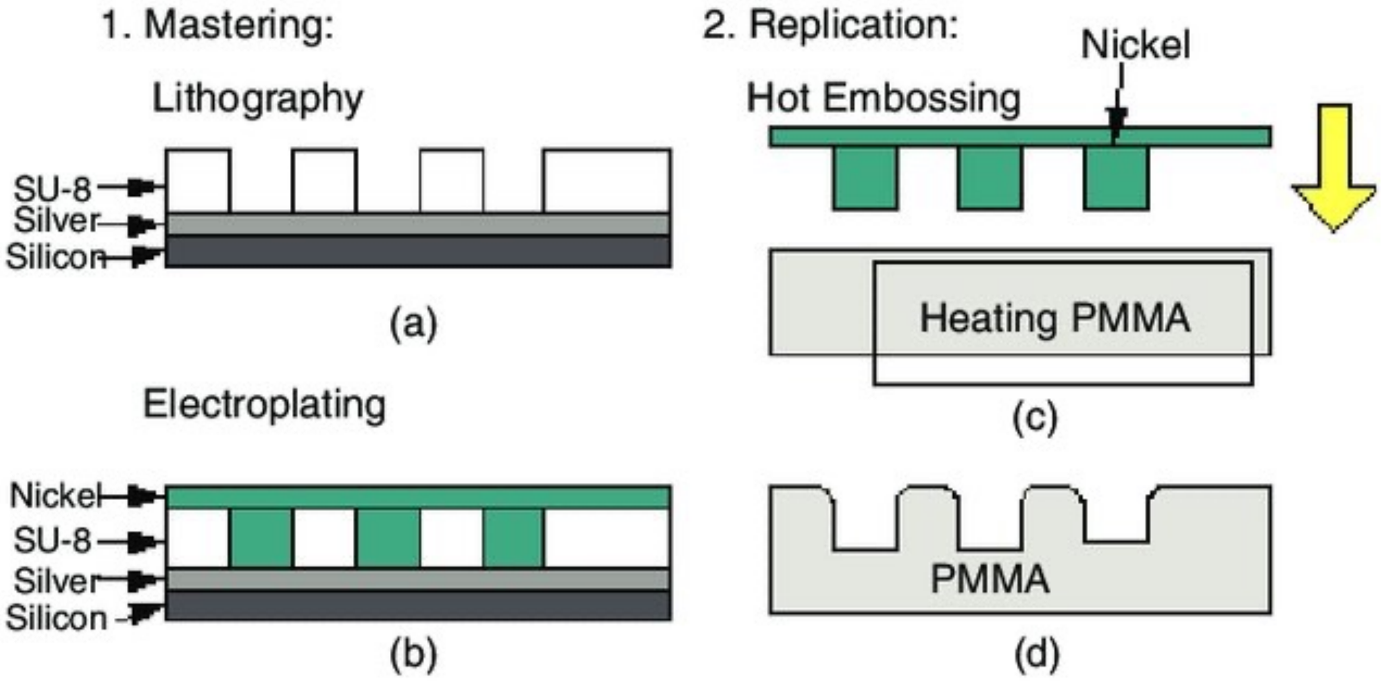
- Soft lithography
  (no limitation as for clean room)
- Easy and chip (low production costs)
- Biocompatible and transparent
- Replication accuracy/resolution:
  100 um (routine) – 10 nm

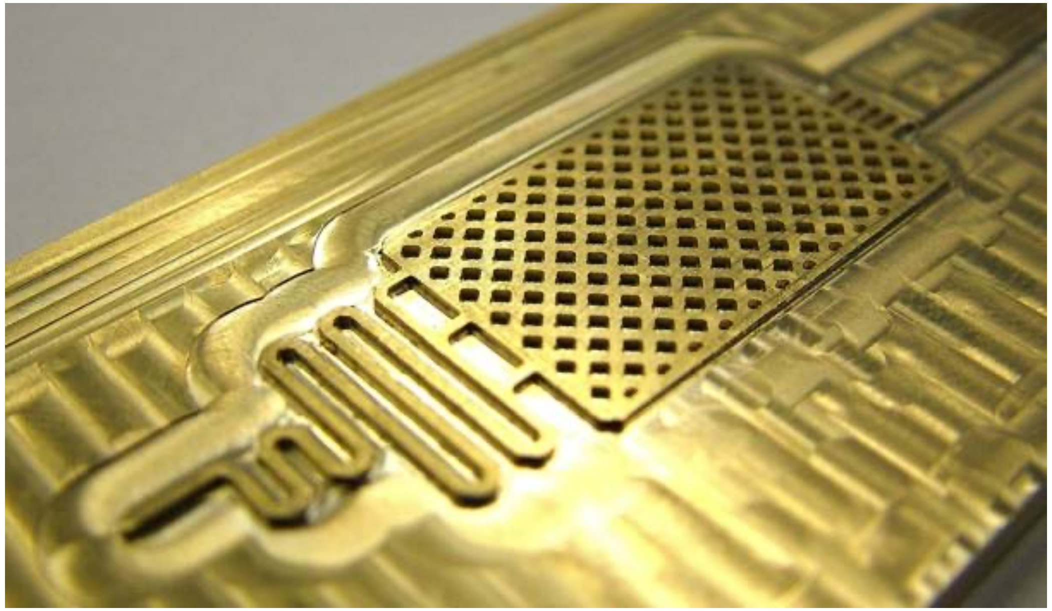
- Issues?
  - Gas-permeability and hydrophibicity
  - Inertness and extensive adsorption



Shanti A et al. (*Pharmaceutics*, 2018)

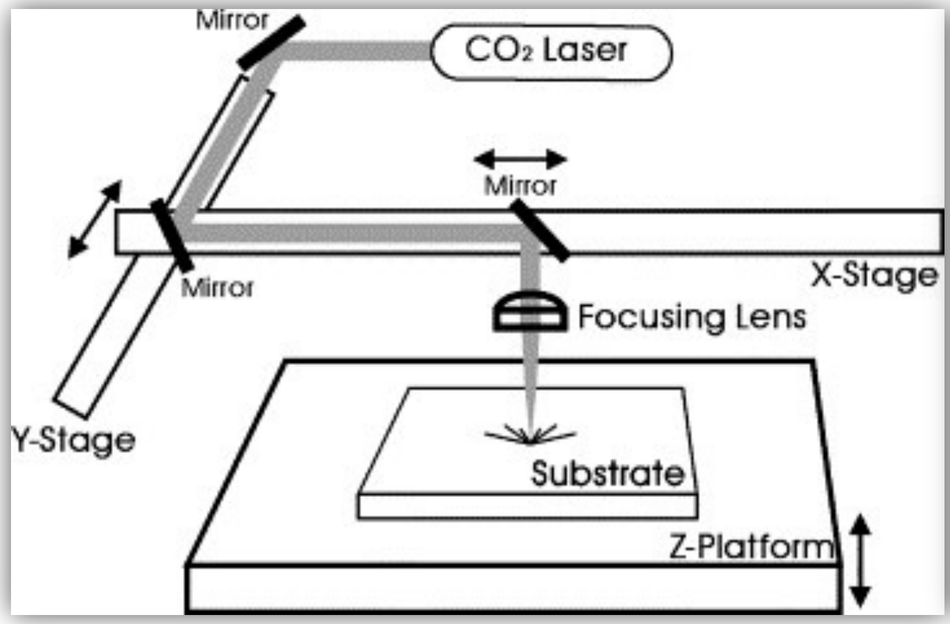
3. Hot embossing

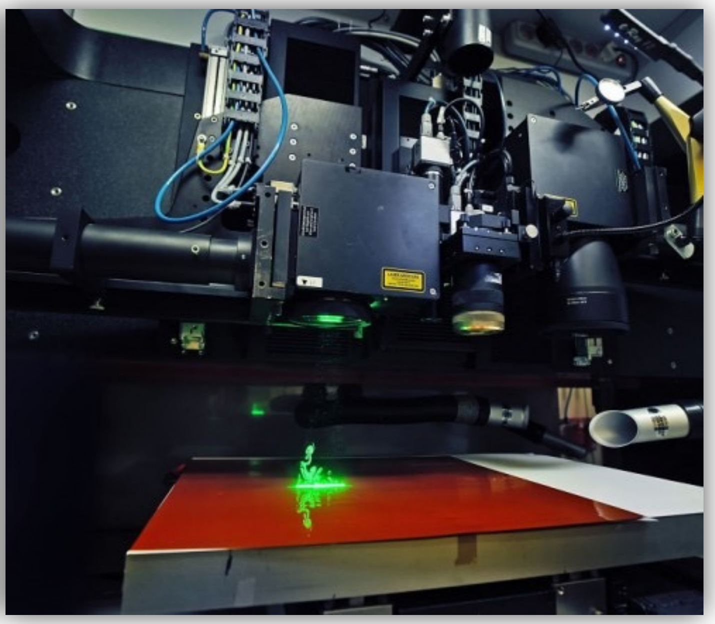


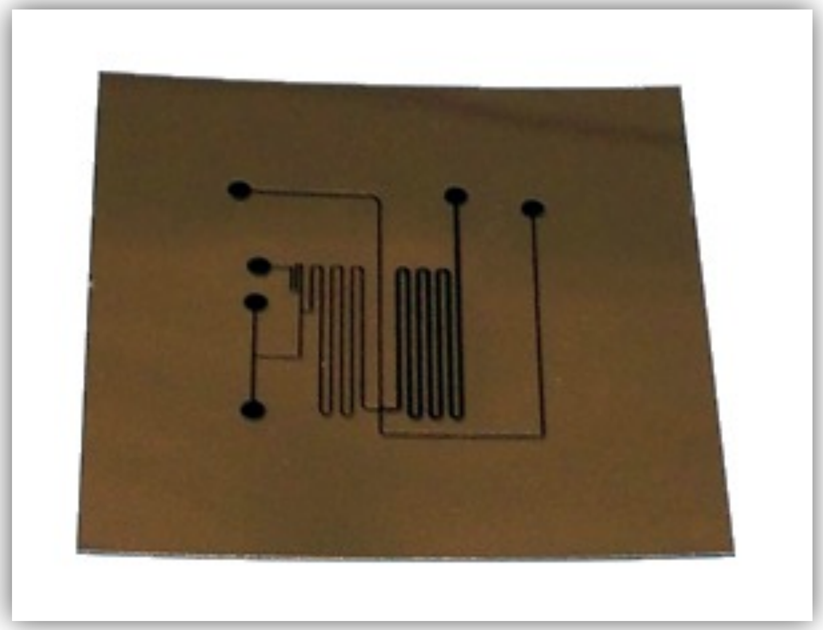


Wen J Li et al. (*Conference on BioMEMS and Nanotechnology*, 2003)

4. Laser Machining (rapid prototyping)

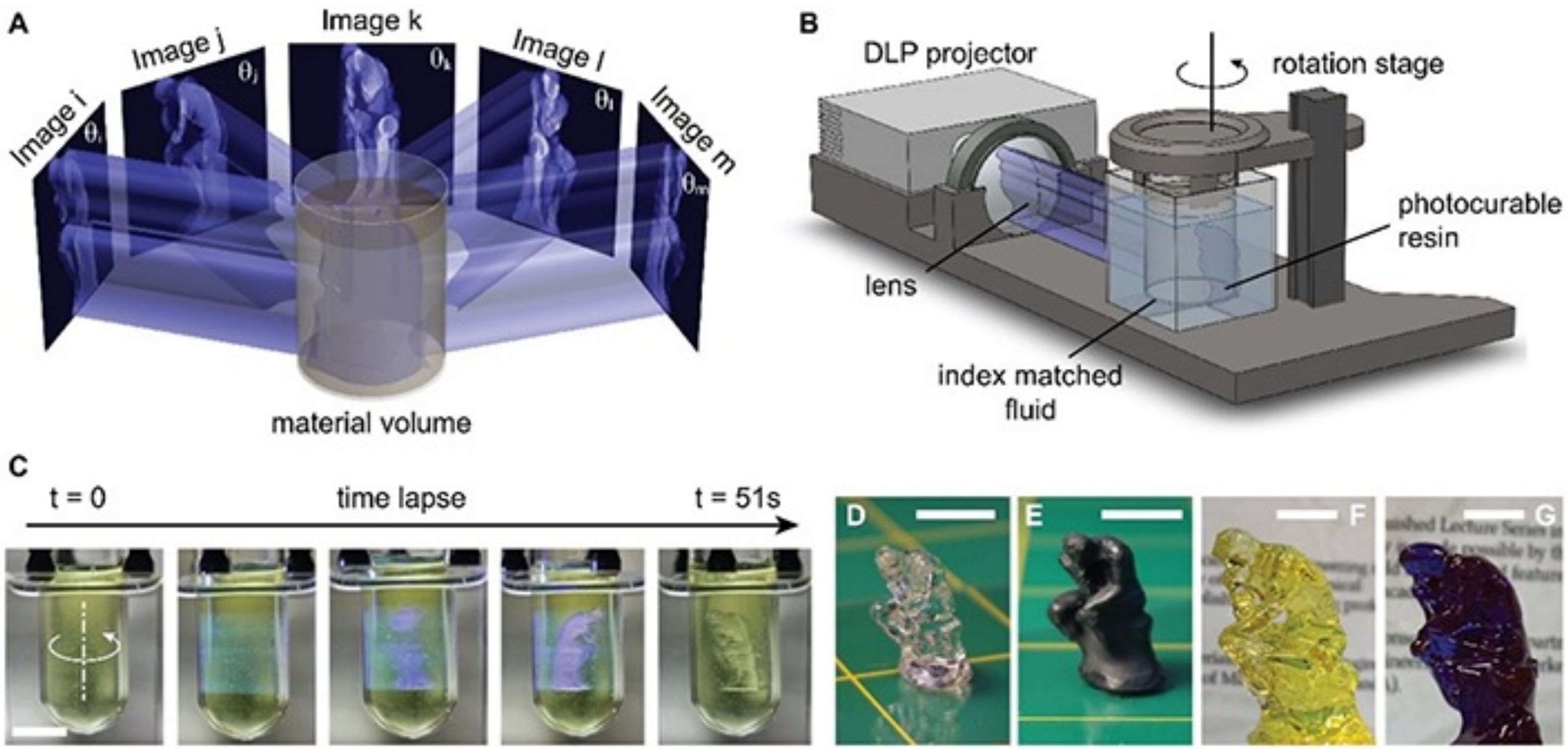


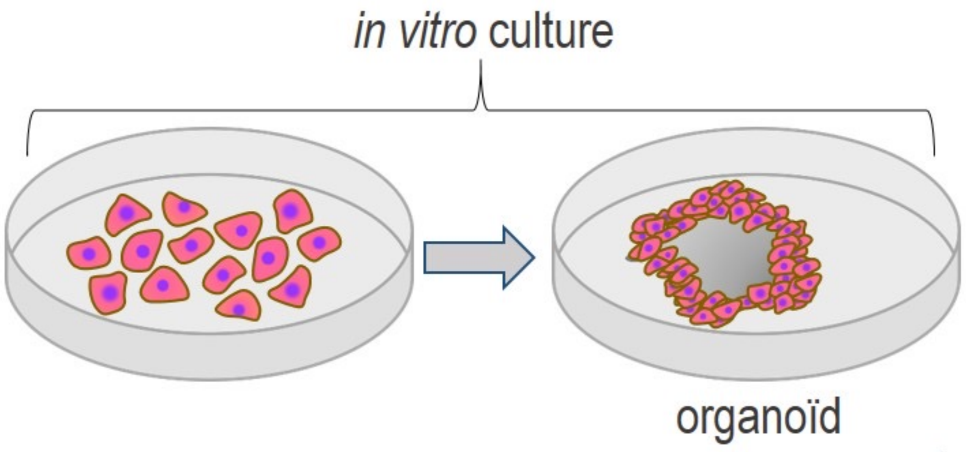




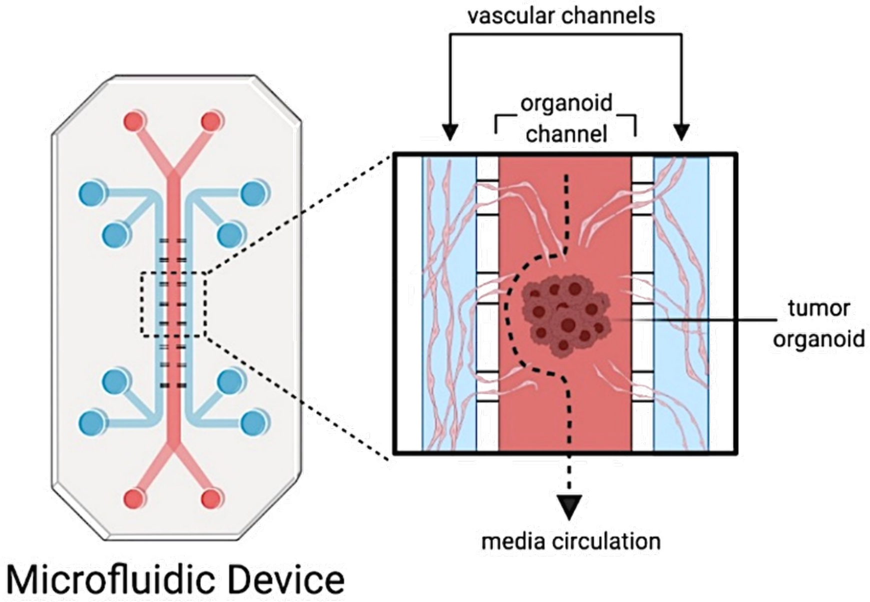
5. Bioprinting

- Independent of bioink viscosity
- Fast (less than a minute):
    - less photoinitiator;
    - better cell viability;
    - Speed allows for multiple iterations.
- Excellent resolution:
    - approximately 40 μm, regardless of the size of the object to be printed;
- Minimal contamination risks:
    - Prints on sealed, autoclavable glass vials.
- Suitable for vascular structures





VS



Why the Micro(fluidic) scale?

10 reasons illustrated by:

- Models/basic physics
- Downscaling/dimensionless numbers
- Examples

1. Laminar flow

- **In microchannels, flow is laminar**
- Reynolds number (Re):
  - ratio inertial/viscous forces
  - Measure for the transition from laminar to turbulent flow
  - Definition:

$$Re = \frac{\rho \, v \, D_H}{\mu}$$

  - For a squared ducted:

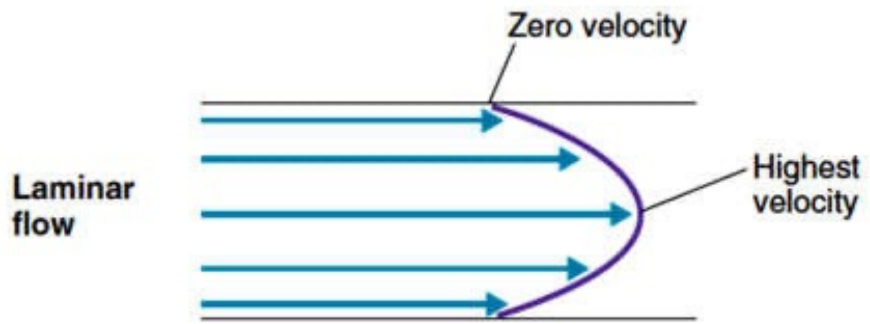
$$D_H = \frac{4 \, a^2}{4a} = a$$

v average speed

D_H characteristic length or hydraulic diameter

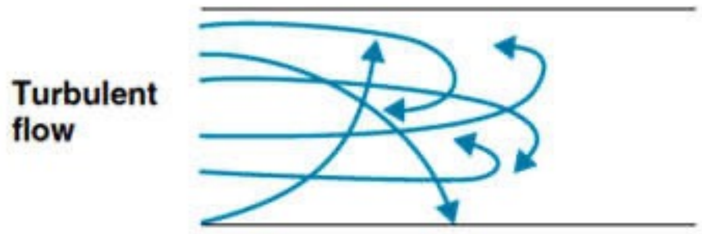
a cross-sectional area

P wetted perimeter



Well defined streamlines
(velocity of a given point does not vary over time)

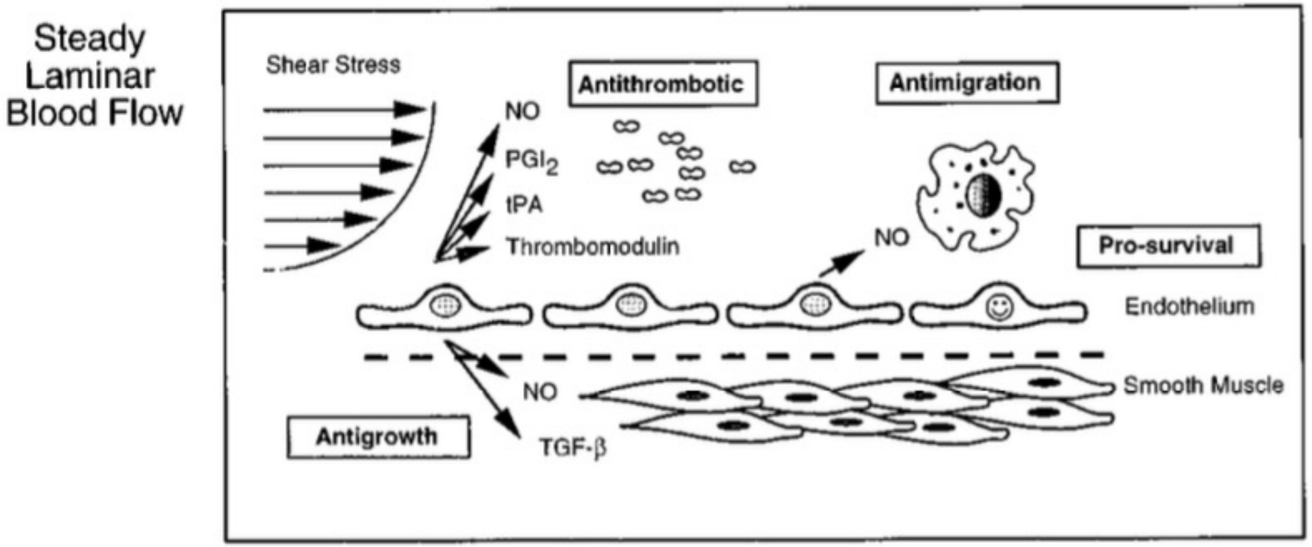
unstable streamlines



Laminar if Re < 2000 and turbulent if Re > 4000

Example of laminar flow relevance in Biology

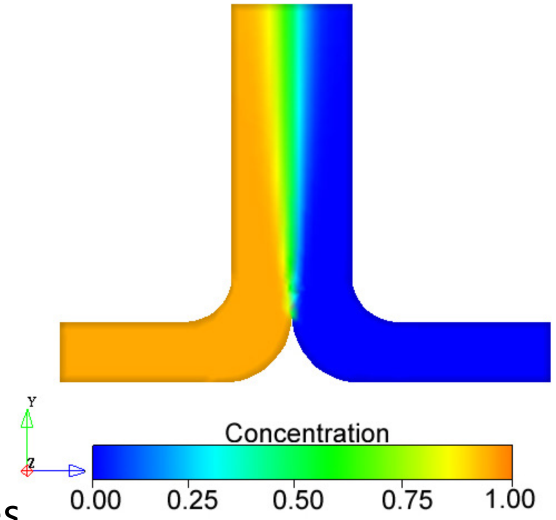
- Controlled shear stress regulates cell beahiour:
  - **Steady laminar shear stress** promotes release of **factors** from endothelial cells that inhibit coagulation, migration of leukocytes, and smooth muscle proliferation, while simultaneously promoting endothelial cell survival.
  - → no atherosclerosis.



Traub O et al. (*Arteriosclerosis, Thrombosis, and Vascular Biology*, 1998)

2. Diffusion

- Mass transport **by diffusion is a slow process**, but on micrometer scale still fast enough



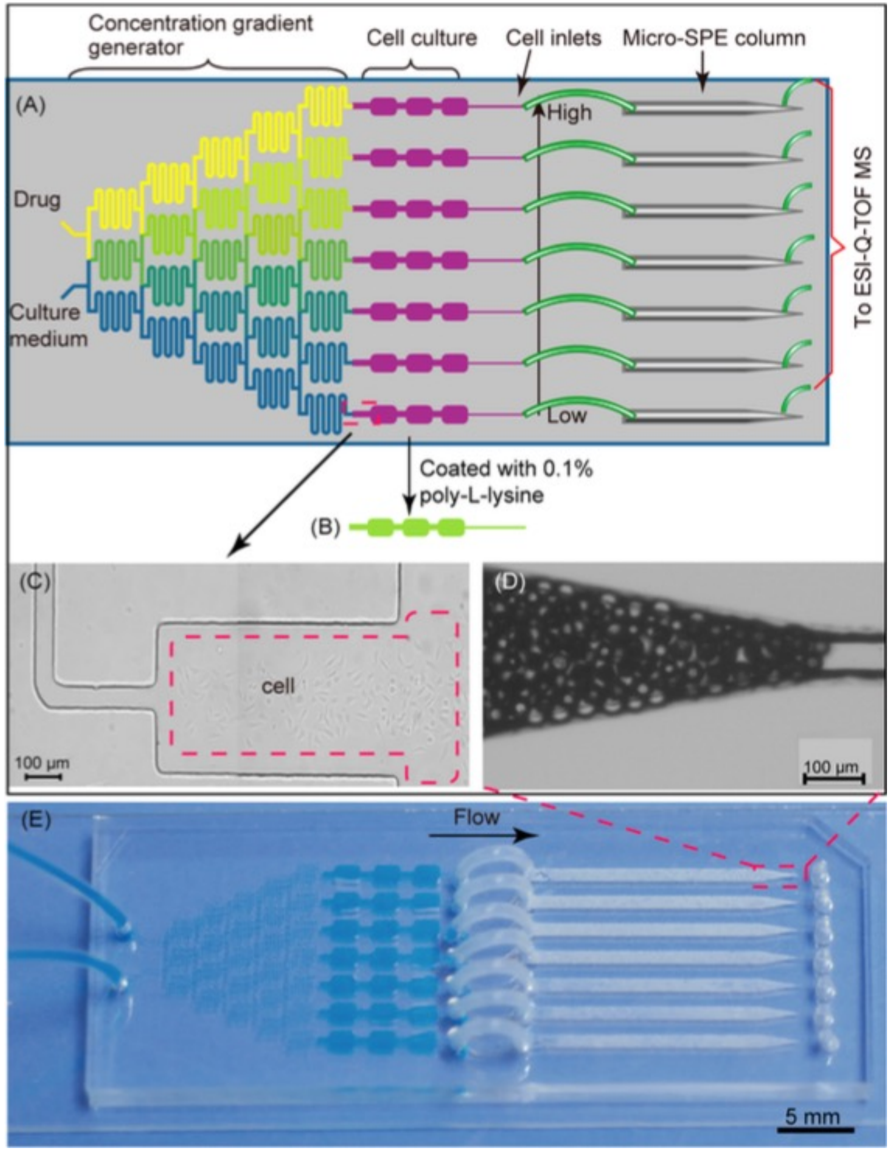
$$d = \sqrt{2\,D\,t}$$

d: distanced travelled by diffusion (m)
D: diffusion coefficient (10^{-9} m^2/s typical for small molecules,
t: time (s)

d	t
45 um	1 s
63 um	2 s
100 um	5 s
500 um	2 min
2.7 mm	1h

Interest: screening of new drug candidates
Odijk et al. (*Biosensors and Bioelectronics*, 2010)

3. High throughput



Gao D et al. (*Analytical Chemistry,* 2012)

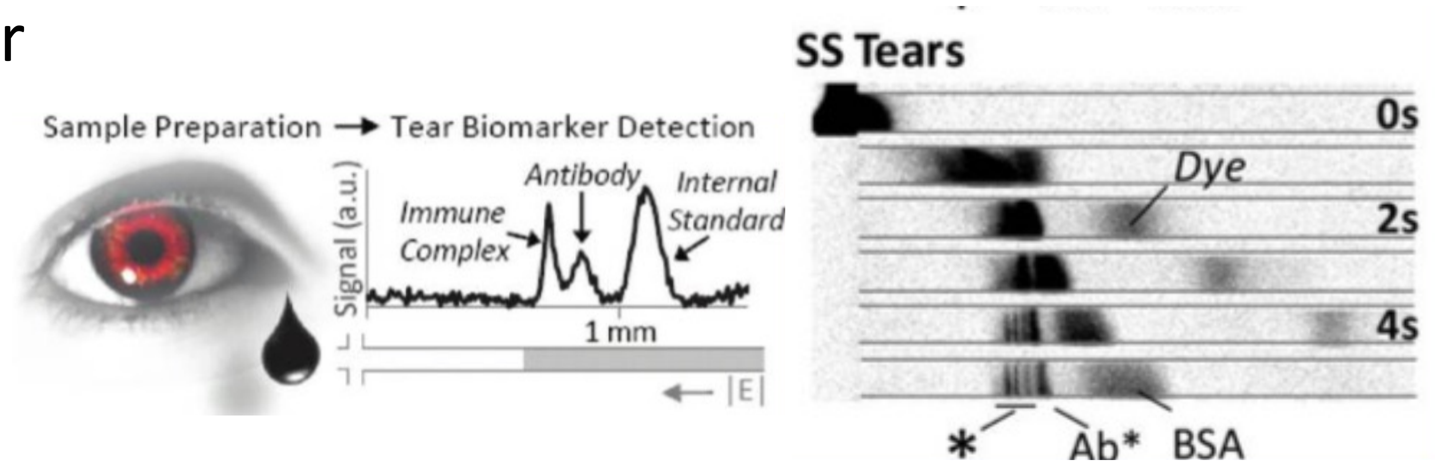
4. Less reagent consumption

- Less reagents → less costs
- But also less waste

→ Especially important for

- Expensive samples
- Small volume samples
- Toxic waste products

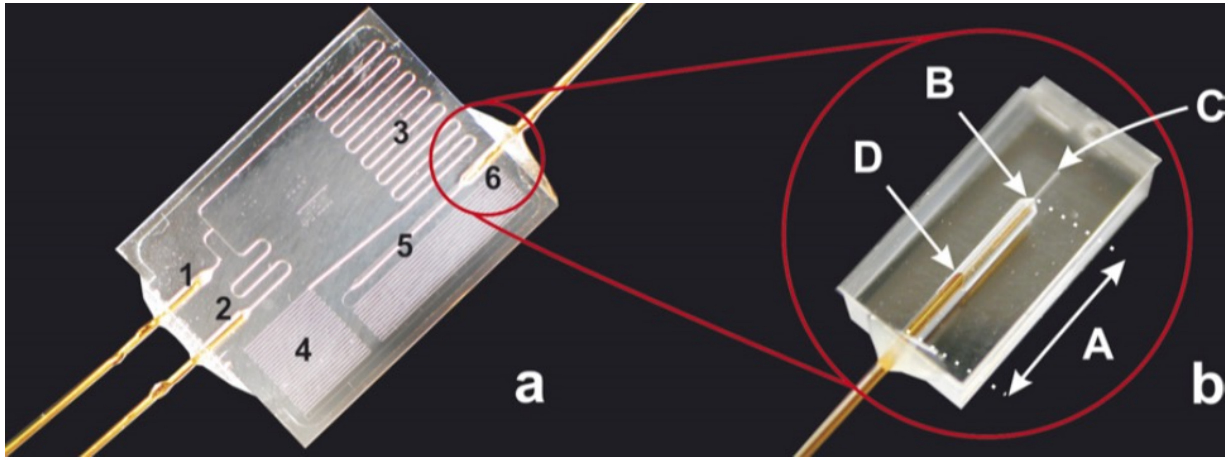
Example: *Lactoferrin (Lf)* is a tear-specific biomarker for *Sjögren's syndrome (SS),* a serious systemic autoimmune disease currently diagnosed through rudimentary surface chemistry measurements and an invasive lip biopsy.



Karns K et al. (*Analytical Chemistry,* 2011)

5. Safety

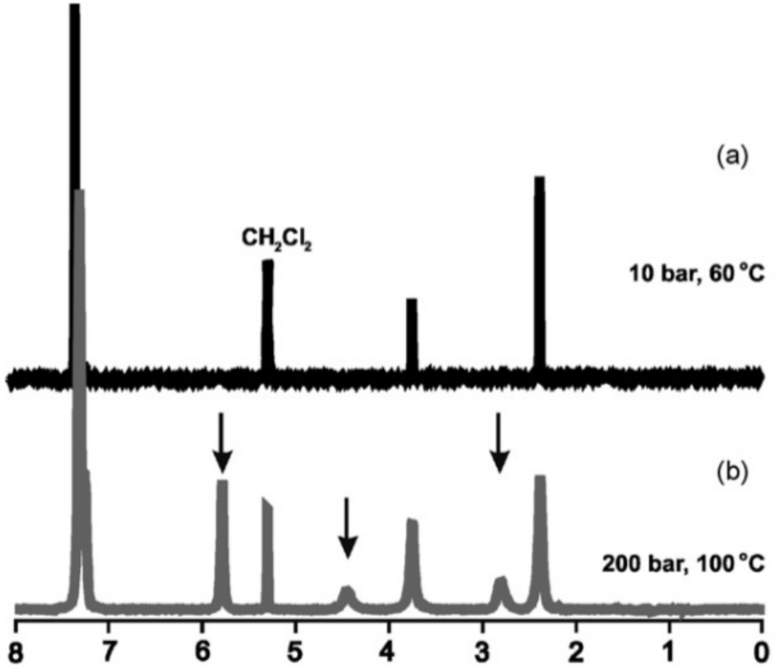
- Enhanced safety because of lower energy stored:
  - At high pressures, a microfluidic plateform is only noticed as a small cracking sound
  - When generating explosive reaction products, the volumes involved are too small to cause damage
  - Very toxic compounds are only used in small amounts, so less health risks due to gases, etc.



Tiggelaar RM et al. (*Chemical Engineering Journal*, 2007)

(1) (2) (3)

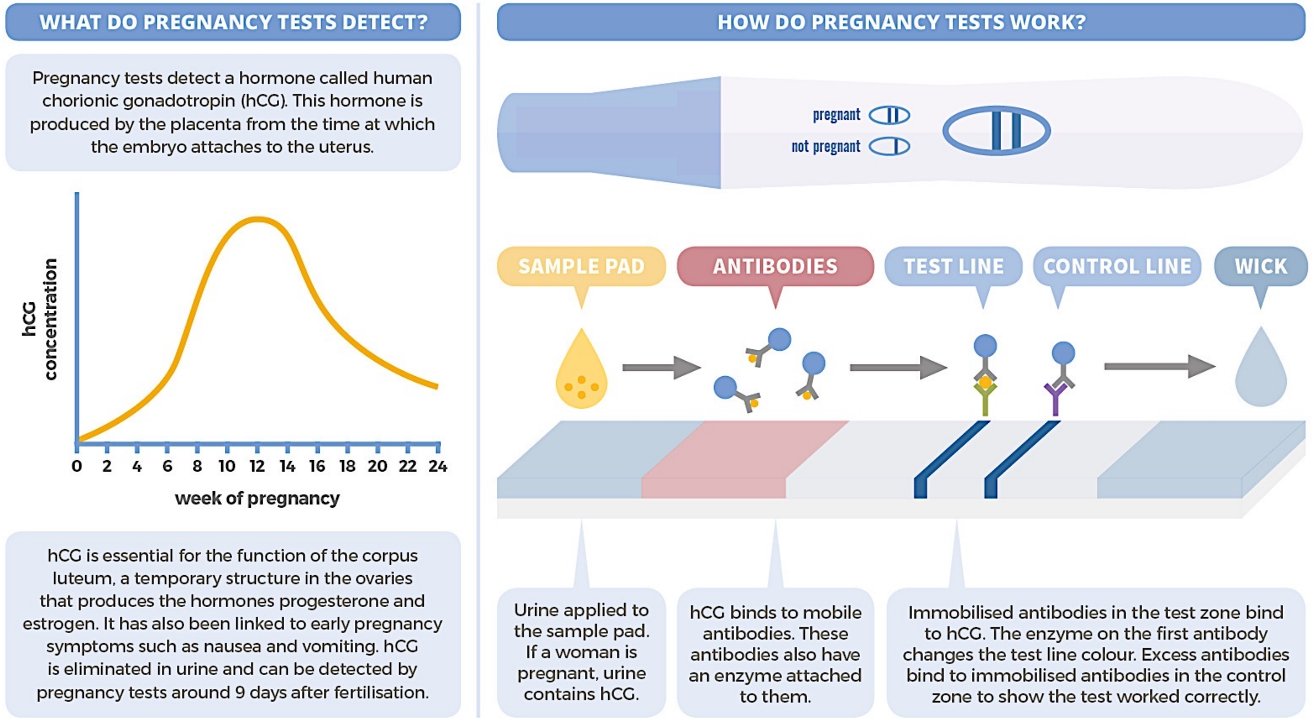
Carbamic acid formation by reaction of *N*-benzyl methylamine (α) and CO_2 (β).



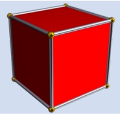
6. Portability and disposability

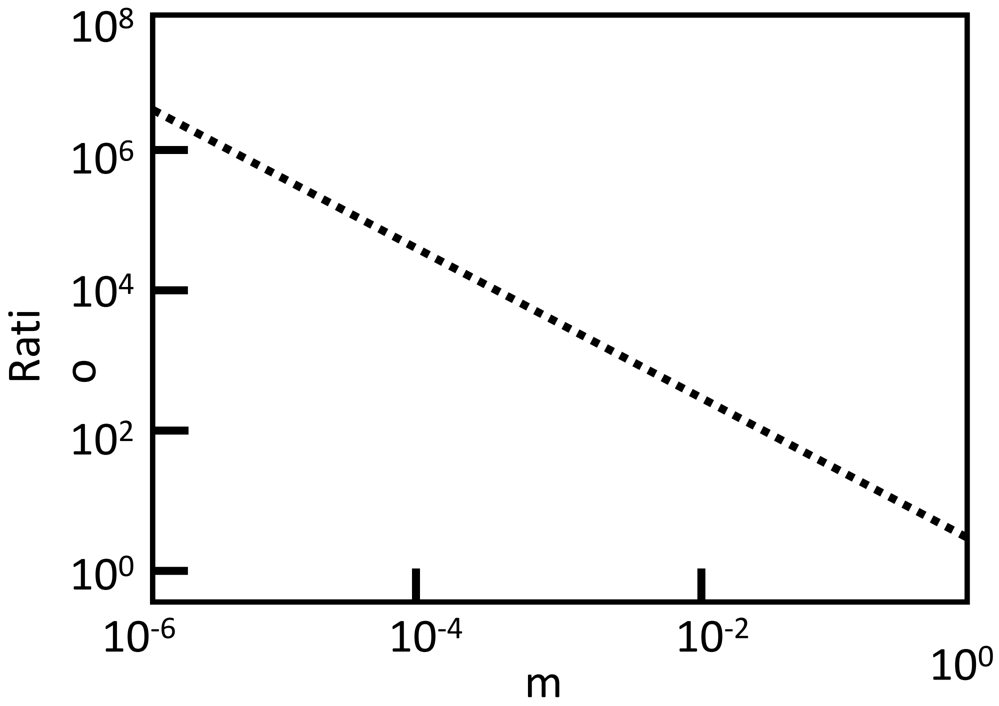
- Disposability: if the chip is only few CHF there is no need for expensive cleaning after measurement

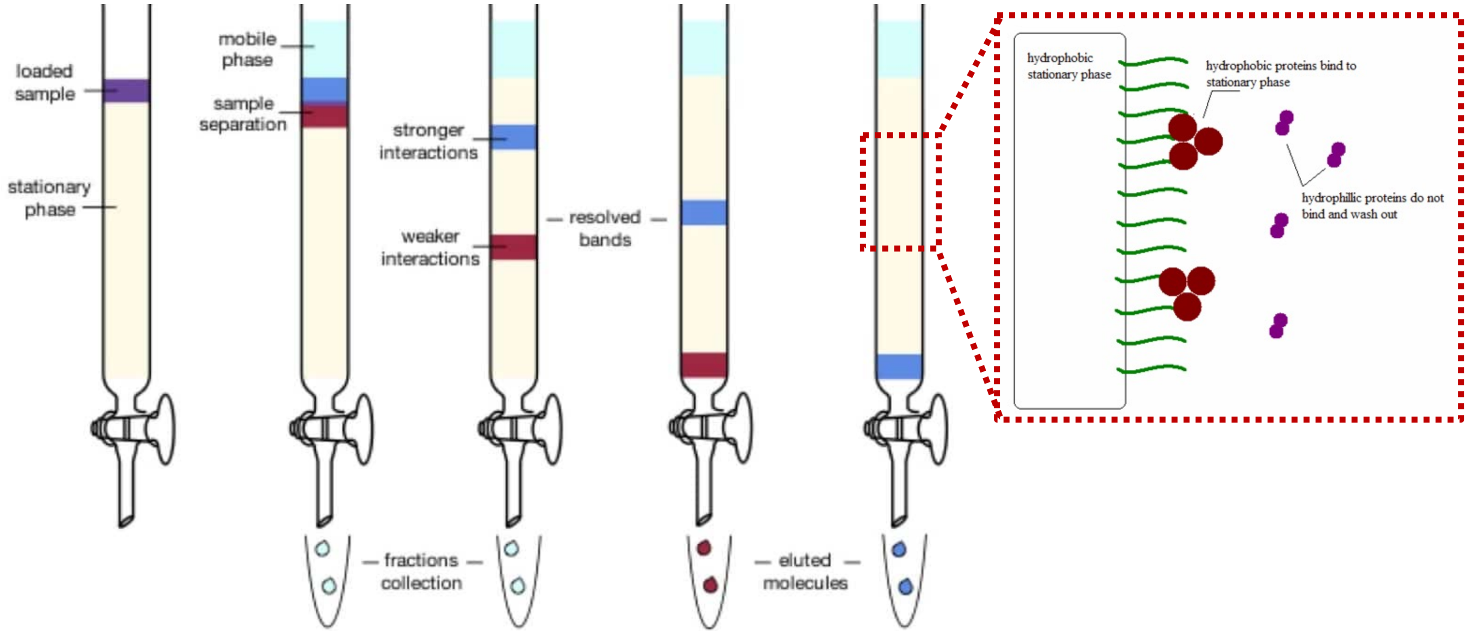
  → Important in the medical applications for contaminations of other patients



7. High surface-to-volume ratio

Shape		Characteristic length a	Surface area	Volume	Ratio
Cube		side	$6a^2$	a^3	$6/a$

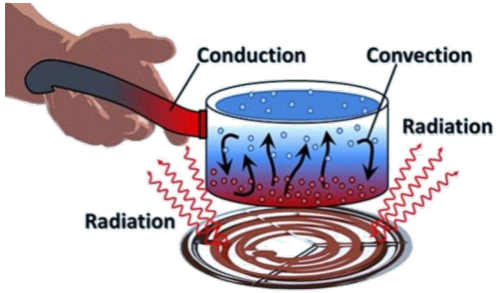




→ A higher ratio implies more interactions with a channel walls

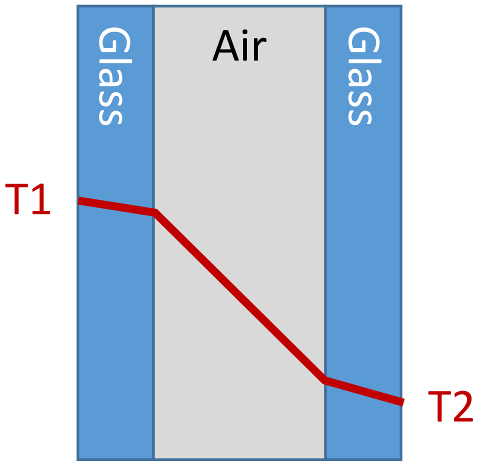
8. Better and faster temperature control

- Heat transfer by 3 processes:
  - Conduction
  - Convection
  - Radiation

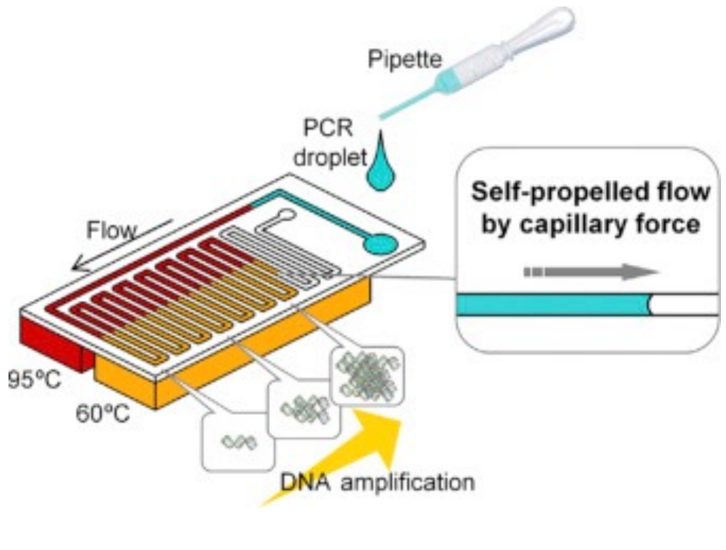


- In microfluidic platforms, heat is mainly transferred by **conduction**, and this is a fast process due to small dimensions

$$\frac{\Delta T}{R_{tot}} = \frac{\Delta Q}{\Delta t} \qquad R_{tot} = 2\,\frac{d_{gl}}{k_{gl}A} + \frac{d_{air}}{k_{air}A}$$

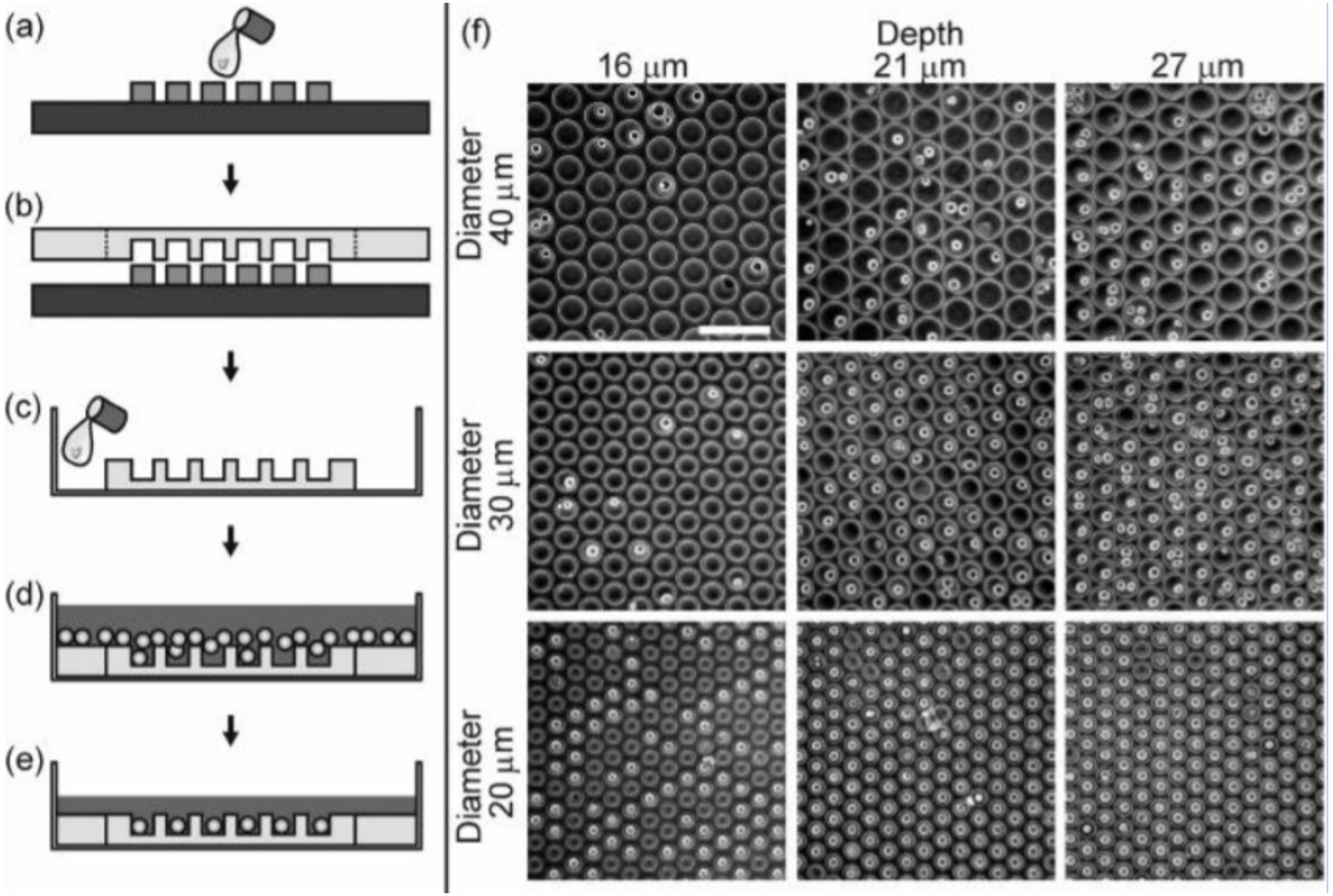


Example of microfluidic PCR



Ali Shahid et al. (Bioelectronics and Medical Devices, 2019)

9. Single Cell Analysis

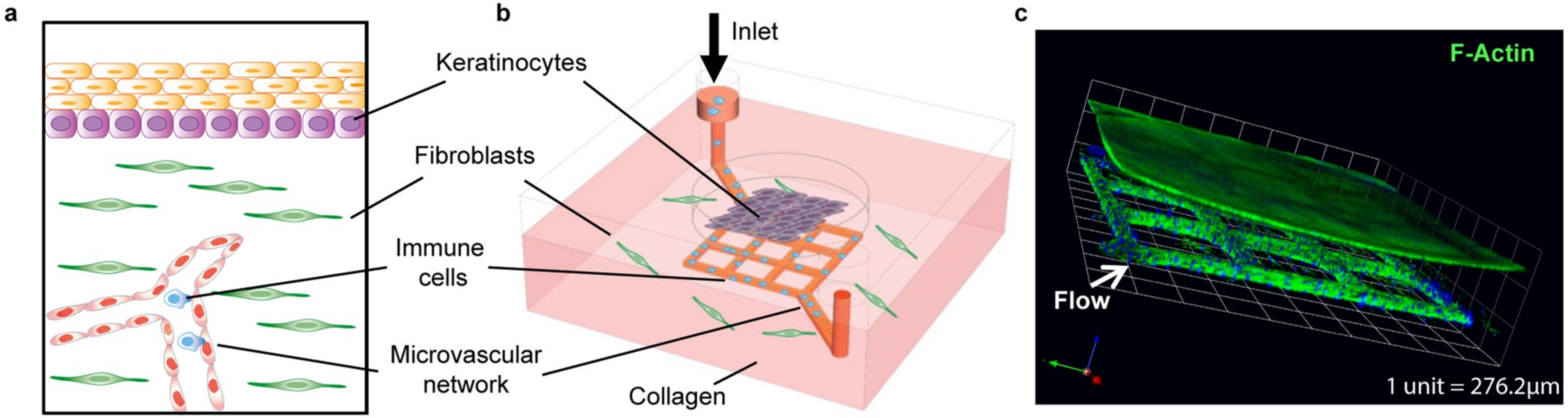


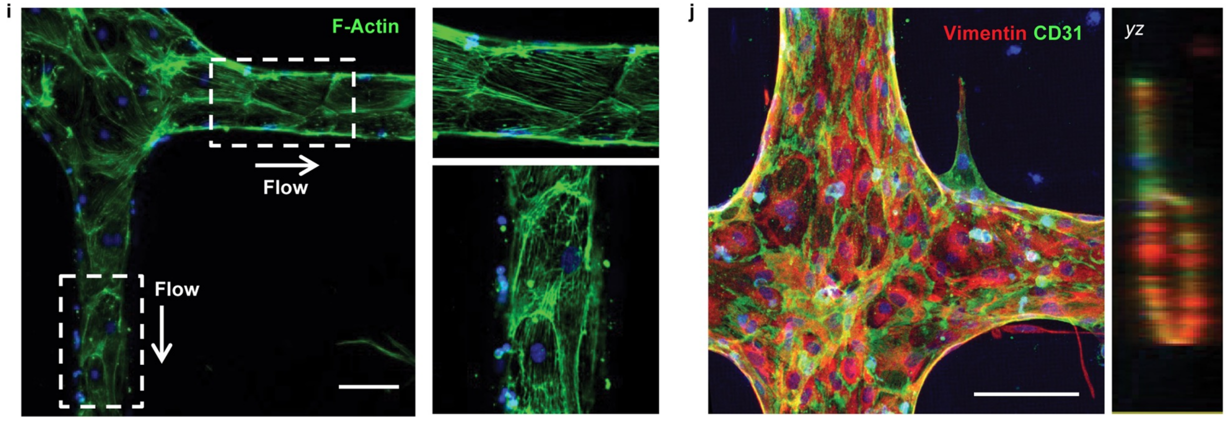
Rettig et al. (*Analytical Chemistry,* 2005)

- Track the changes (missed by bulk sequencing)
- Example: Study the genetic evolution of cancer, as cancer cells are constantly mutating; heterogeneity of cell populations can be observed using single cell sequencing

10. Complex tissue organization

«Skin-on-chip» mimicking a stratified epidermis with a dermis perfused by a microvascular network





Sun S. *et al.* (*Nature Communications*, 2022)

Outline

- Part 1
  - What is an organ-on-chip system, its origin and advantages
  - Fabrication methods
  - Why the microfluidic scale?
- Part 2
  - Relevance of mechanobiology
  - Biomechanics in microchip systems
  - Cell response and Analysis (quantification: western blotting, immunochemistry)

Questions?

End of Part 1