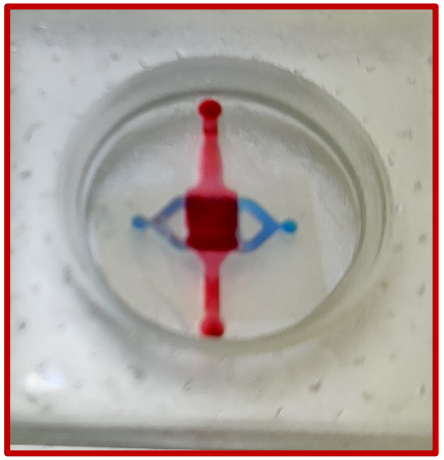

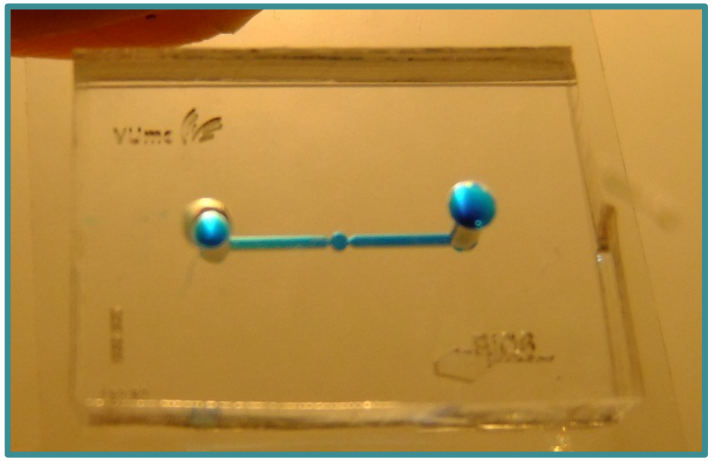

Designing microfluidic systems for cell biology

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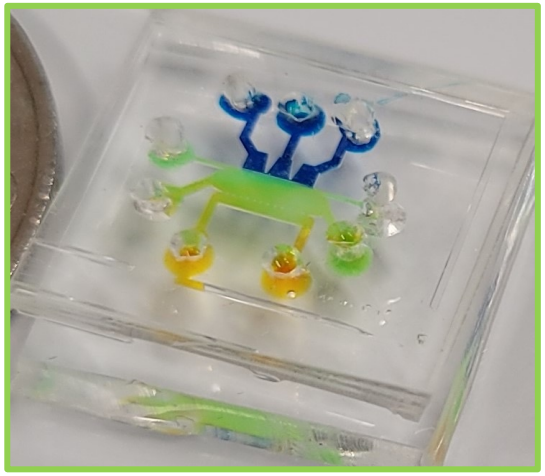
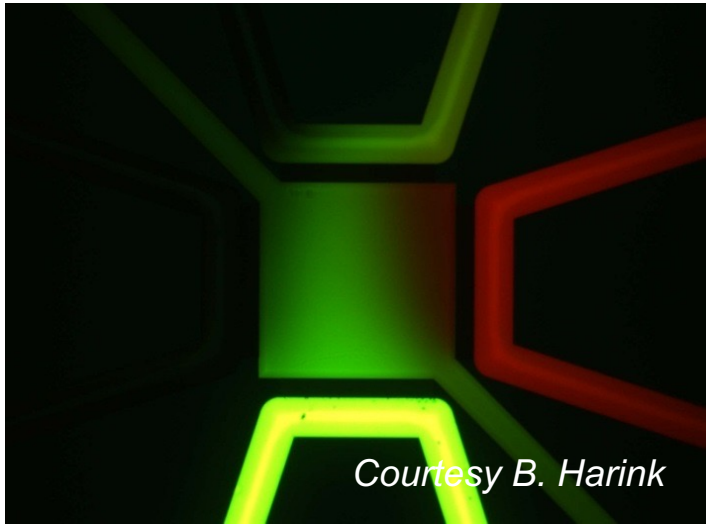
UNIVERSITY OF TWENTE.

Content and scope of the course

- **Motivation**:
  - Why using microfluidics for cell studies?
  - Unique features offered by microfluidics for cell studies



- Important parameters to consider when designing microsystems for cell studies / experimentation
  - Environment / format of the culture
  - Material
  - Pumping
  - Surface chemistry / topography
  - Gradients

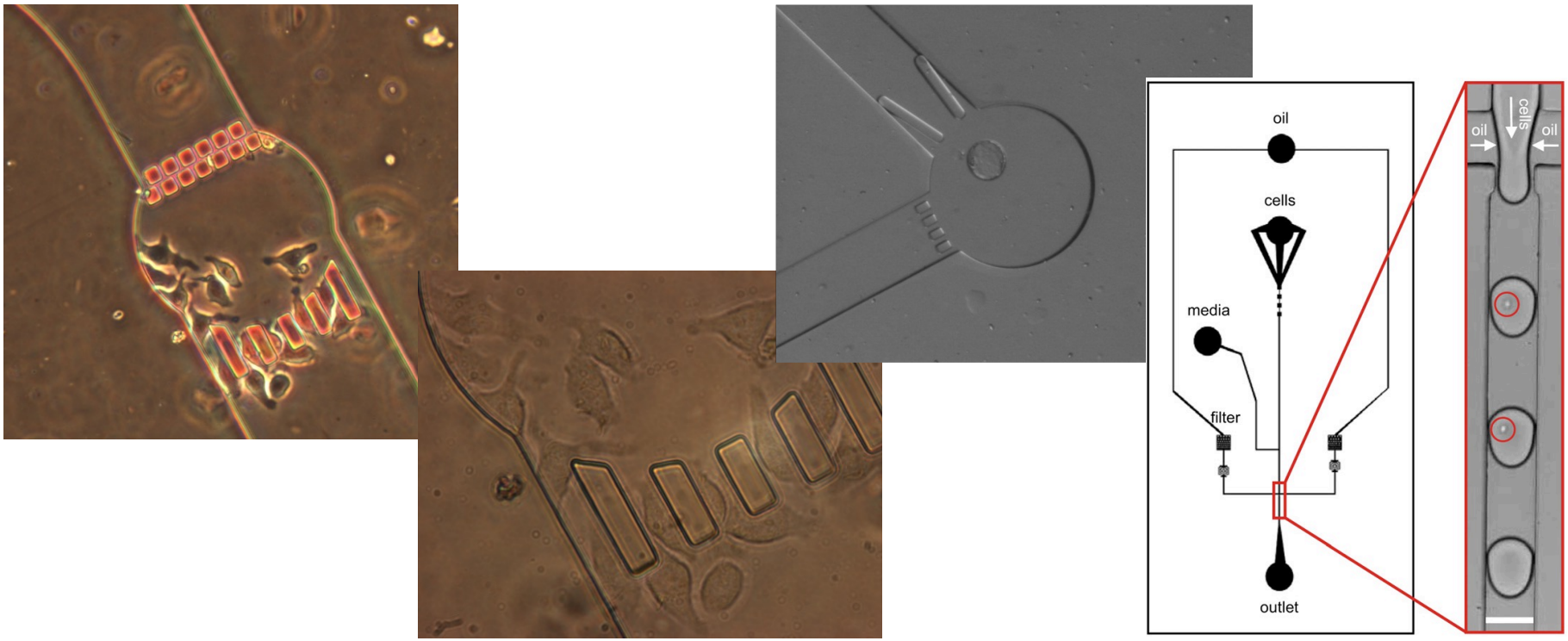


I. Motivation
Why using microfluidics for cell studies?

Why using microfluidics for cell studies?

Single cell experimentation

- **Size** of the system comparable to the size of cells (10-100 µm)
- **Cells trapped** thanks to a microstructure: cells easy to “follow”
  - ⇨ Tracking of **events** at the single cell level
  - ⇨ Suitability to work with rare cells
  - ⇨ New experimental opportunities!!



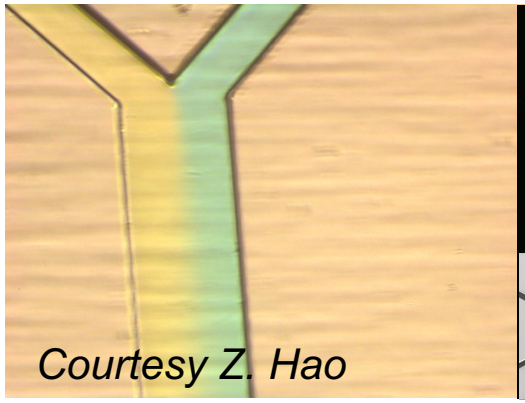
Why using microfluidics for cell studies?

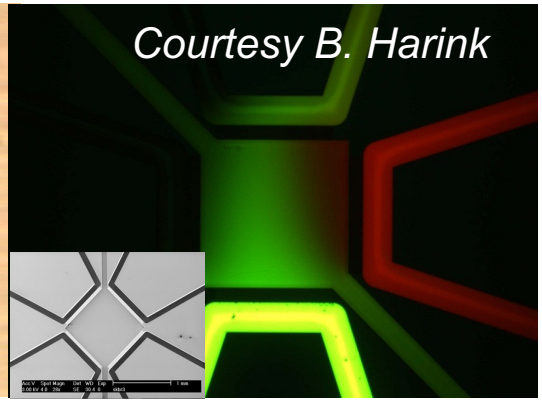
- **Micrometer size ⇨ Highly controlled microenvironment**

- **Laminar** character of the **flow**

⇨ Predictable and controllable flow

⇨ Possibility to create gradients





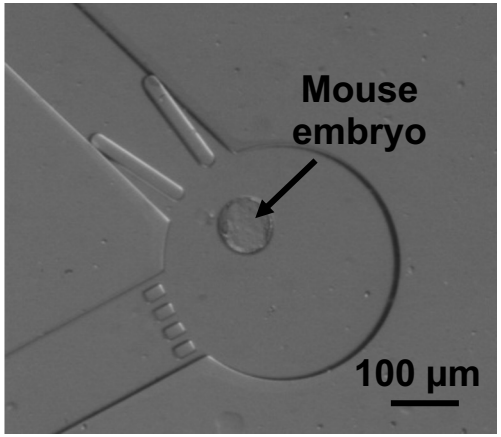
- **High surface-to-volume ratio**

⇨ Efficient surface-based exchange phenomema (*e.g.*, heat & gas exchange)

⇨ Controllable and tuneable physical microenvironment

- **Confinement**

⇨ Close to ***in vivo*** **conditions**

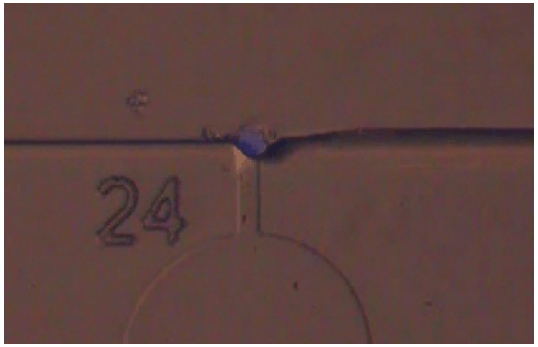


⇨ Advantageous format for culturing cells and creating *in vitro* cellular models

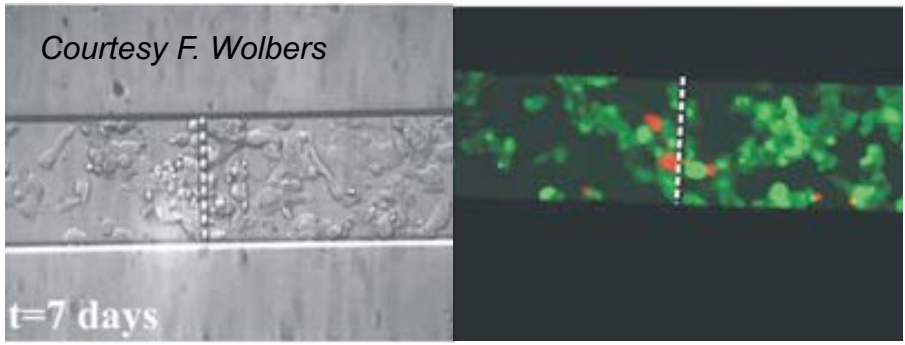
Le Gac et al., *Tr. Biotech.*, 2008 ; Harink et al., *Lab Chip*, 2013; Picollet D'hahan et al., *Tr Biotech.*, 2021.

Microfluidics and cell studies

System complexity

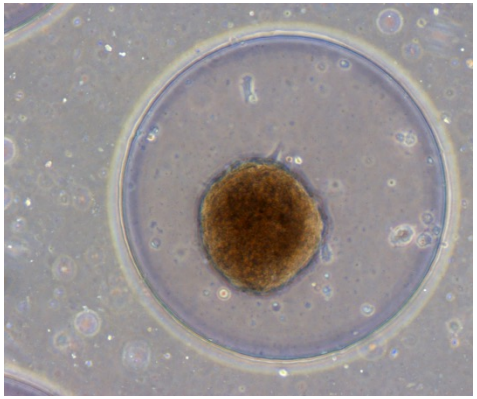


0D-1D
Single cell

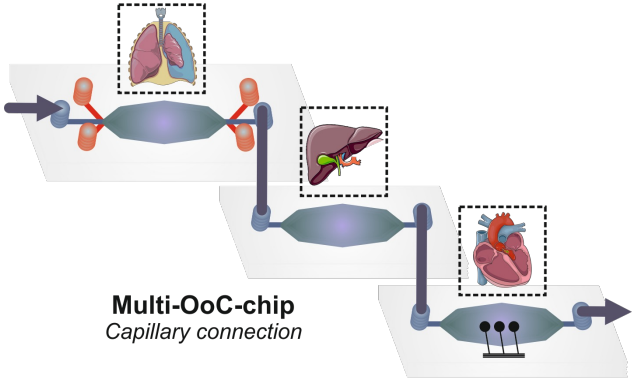


2D
Monolayer

Tissues

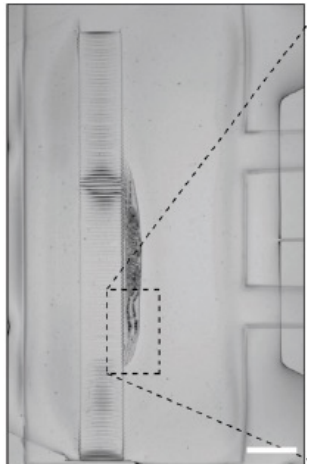


3D
Organ-on-a-chip



Picollet D'hahan et al., *Tr Biotech.*, 2021.

Small Animals



Rohde, PNAS, 2007

Applications of microfluidics for cell studies

Single cell studies

- *Single cell analysis*

Retrieval of specific molecules (DNA, RNA, targeted proteins) inside a cell and on-line analysis (on-chip or off-chip)

Single cell imaging or characterization (electrical, mechanical, etc.)

- *Single cell engineering*

Cell electroporation, intracellular injection, cell fusion

- *Single cell treatment*

Exposure to chemical/electrical stimuli (possibly followed by cell analysis)

Monolayer (2D) studies

- Cell culture
- Cell electroporation
- Drug screening

Tissue studies (3D)

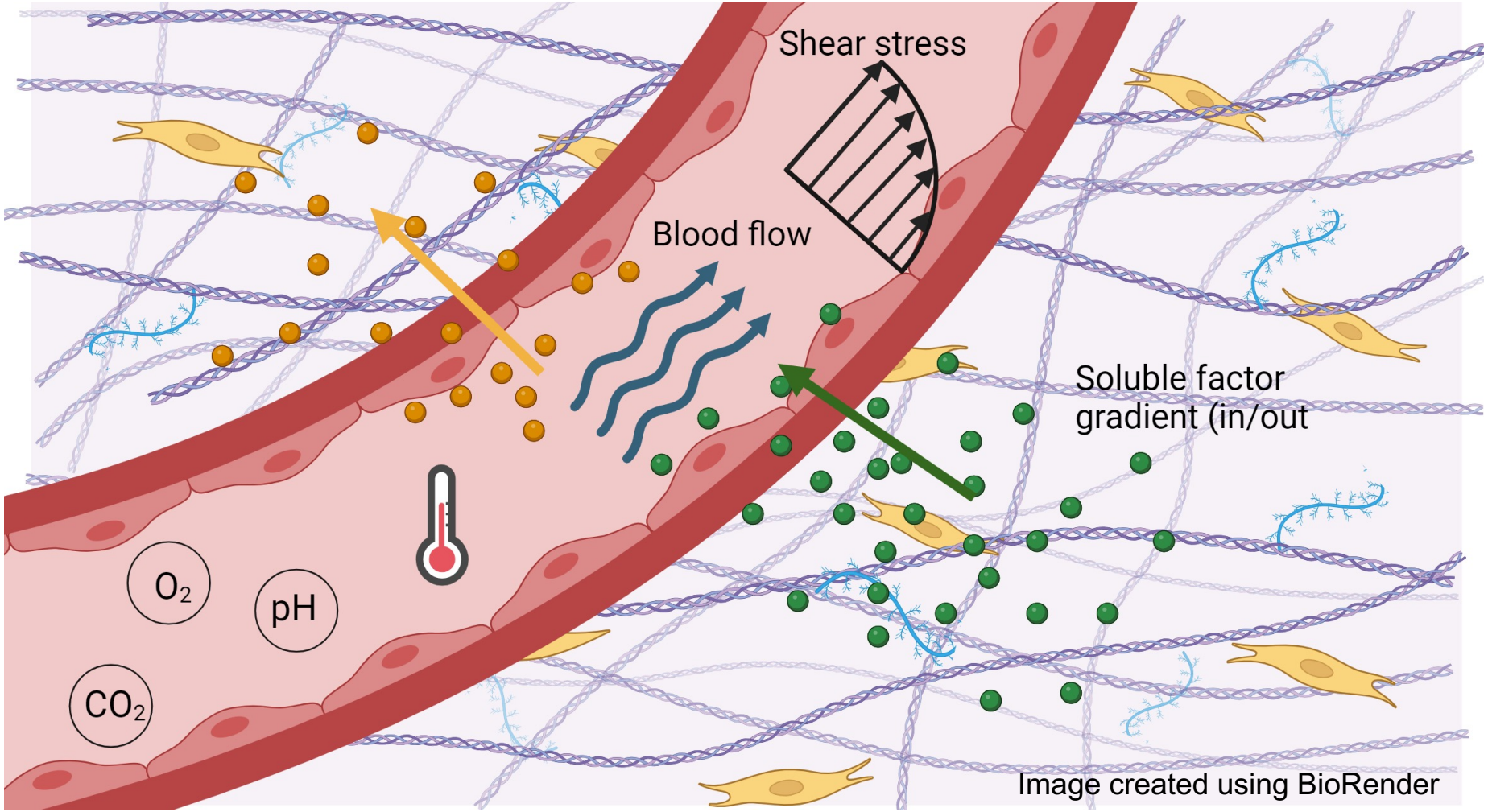
- Tissue engineering / Cell culture
- Fundamental studies on tissue formation (angiogenesis, differentiation)
- Drug screening, metabolism studies
- Organ-on-chip platforms

II. Cell culture and microfluidics: Important parameters to consider

Important parameters

- **Cell microenvironment**
  - Physical and chemical parameters (TºC, gas %, …)
  - Mechanical cues – passive (hydrogel properties) *vs*. active (applied forces)
- **Materials**
  - Cell-friendly (biocompatible), cheap, easy fabrication
- **Liquid flow / Perfusion**
  - No hyperphysiological shear on cells (death), easy operation
- **Surface properties**
  - Control cell adhesion (adherent *vs*. suspension cells)
  - Control cell behavior (topography, stiffness, chemistry)
- **Gradients**
  - Soluble factors
  - Surface (functionalization; mechanical properties)

The *in vivo* cellular microenvironment



Different contributions: mechanical, biochemical, physicochemical factors

Young et al., 2009

The *in vivo* cellular environment

- **Polarized cells**
  - Cells located at the edge of a lumen in the body, in physiological barriers (epithelial cells)
  - Forming a tight monolayer and establishing tight-junctions

⇨ Culture on **specific substrates, with flow,** to promote cell polarization
⇨ **3D culture** (in an ECM-rich environment)

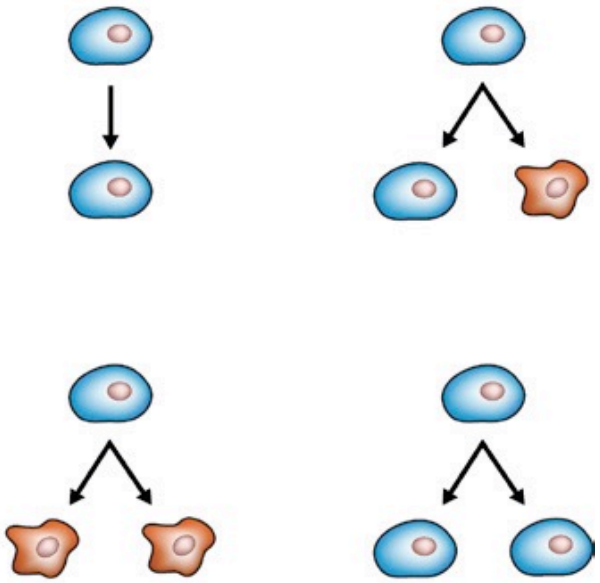
- **Cancer cells**
  - When cultured in 2D, loss of their cancer character……
  - Not only influence of gene mutations, but also influence of external factors (ECM, cell-cell contact).

⇨ Need for **3D culture** platforms and *in vivo*-like environment

ECM = extra cellular matrix

The *in vivo* cellular environment

- **Stem cell**
  - Capacity to differentiate into specialized cell types
  - Self-renewal or ability to divide while remaining undifferentiated



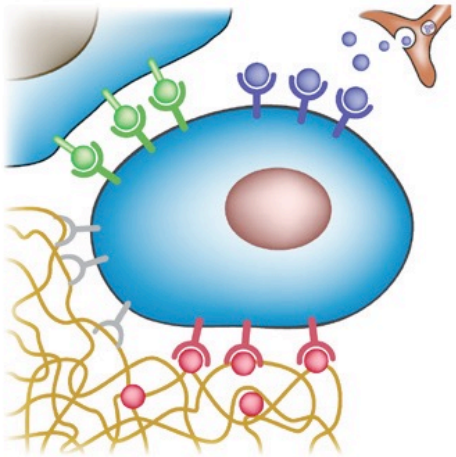
External factors ⇔ fate of the cells

1. Quiescent cell
2. Asymmetric division ⇨ stem cell + differentiated cell
3. Symmetric division ⇨ 2 differentiated cells
4. Symmetric division ⇨ 2 stem cells

- **Stem cell niche**

Cell-cell interactions
- Cadherins
- Cell adhesion molecules (CAM)
- Notch ligands

ECM adhesion
- Fibronectin
- Laminin
- Collagens
- GAGs
- Vibronectin, ...



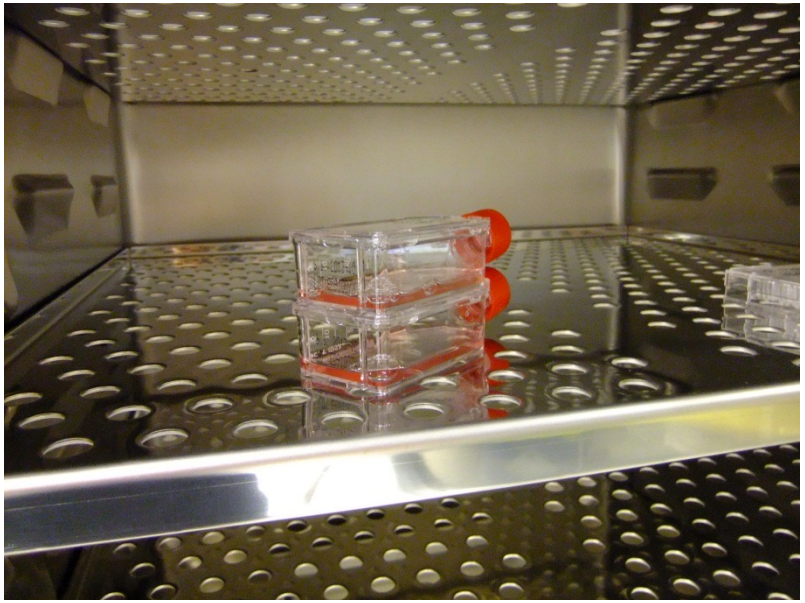
Metabolic cues
- Ions, hormones, ...

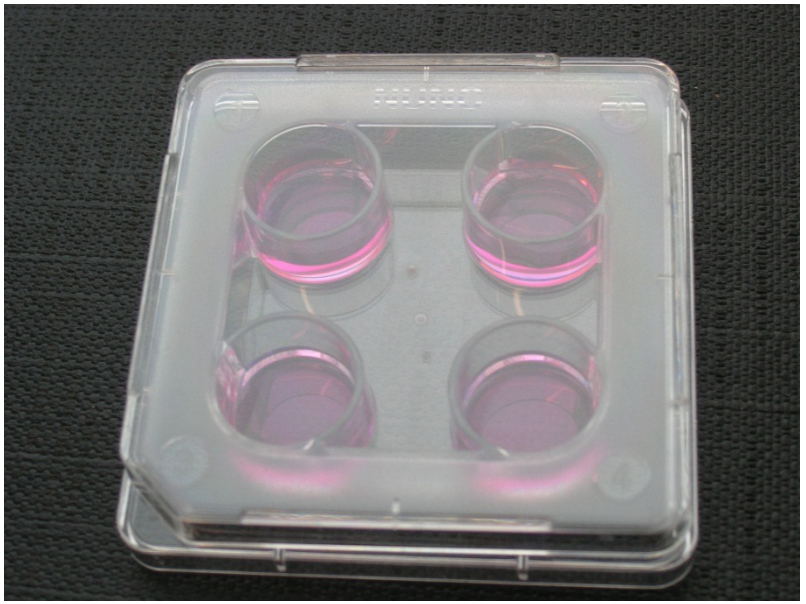
Biophysical aspects
- Substrate elasticity
- Physical forces
- Spatial arrangement, ...

Soluble signals
- Growth factors
- Cytokines
- Chemokines

Kobel et al., 2010;
Lutolf et al. 2009

Conventional cell culture

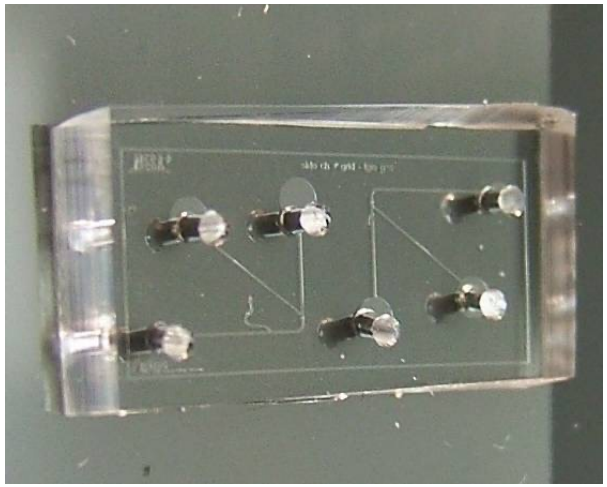


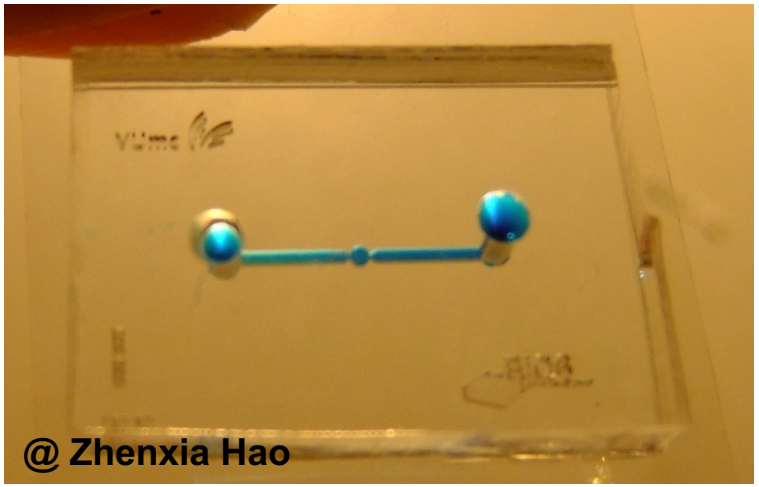


- **Static** culture, punctual medium exchange
- Culture medium: **excess of nutrients**, promoting cell proliferation
- Physicochemical parameters (incubator level): temperature, % CO_2, pH
- Limited flexibility and **throughput** on the experimentation/ culture
- Not suitable to recapitulate the **cell microenvironment**

⇨ **Fully artificial culture conditions**

Microfluidic cell culture





- **Highly confined** environment: *"in vivo* like"
- **Dynamic culture**: continuous medium perfusion, pulsatile delivery, rapid medium exchange, etc…
- Emulation of the *in vivo* **physiological microenvironment** (incl. spatio-temporal variations)
- Generation of **gradients**; biochemical and mechanical stimulation
- Ideal format for higher **throughput** and **parallelization** of the experiments

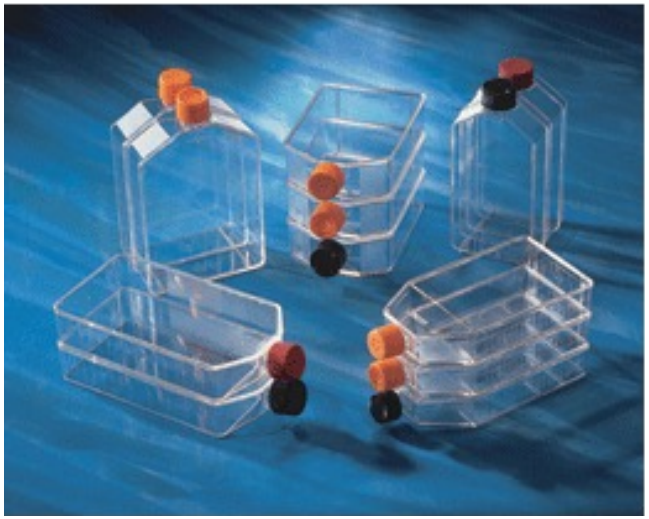
  ⇨ possible screening of simplified culture conditions

⇨ Microfluidics: attractive format in cell biology

Choice of the material(s) for the device

Requirements / wish-list:

- Biocompatible
- Gas-permeable
- Easy processing (no need for a dedicated clean-room environment)
- Cheap (single-use device)
- Optical transparency and low autofluorescence level

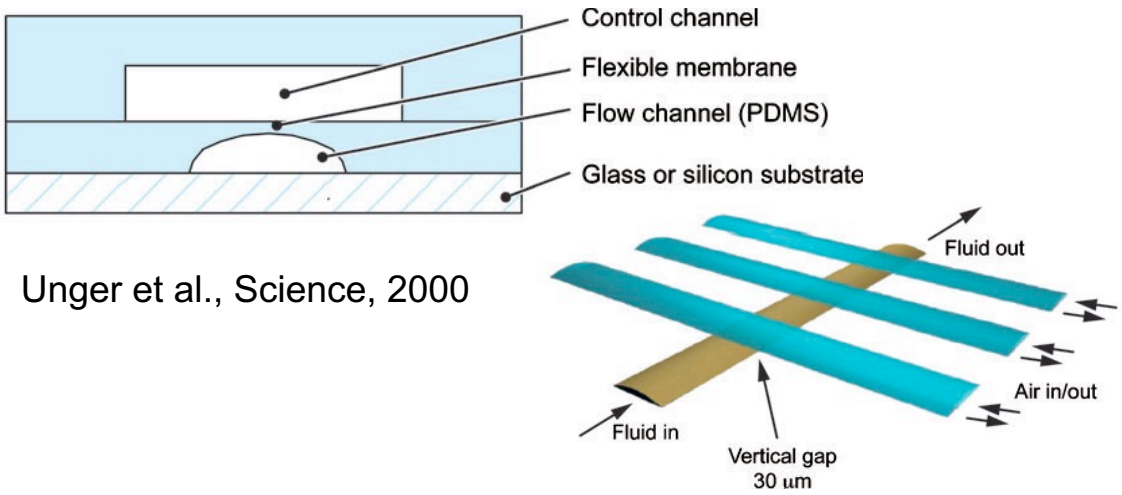


Conventional dishware:
Polypropylene or polystyrene; possible plasma treatment to make them hydrophilic

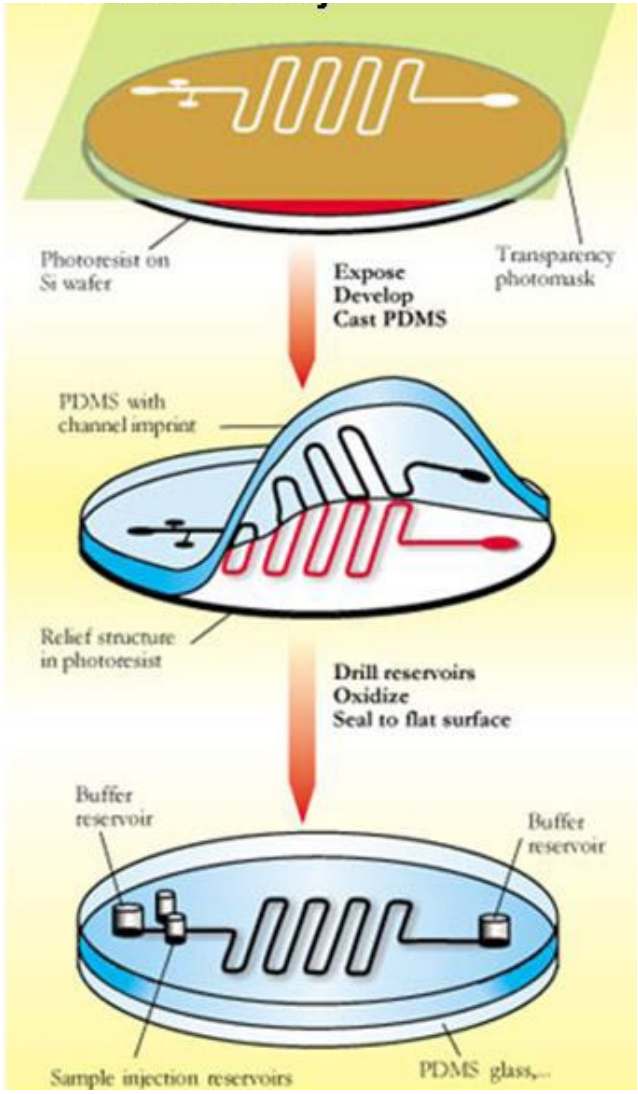
Materials - PDMS

PDMS (polydimethylsiloxane)

- **Predominant** material for microfluidic cell studies
- Cheap, biocompatible, gas-permeable
- **Easy** and **fast** fabrication within a few days
- No need for a cleanroom environment
- Processing using **soft-lithography**: 2D and 3D structures down to low micrometer-size
- Ideal for the realization of **valves**



Unger et al., Science, 2000

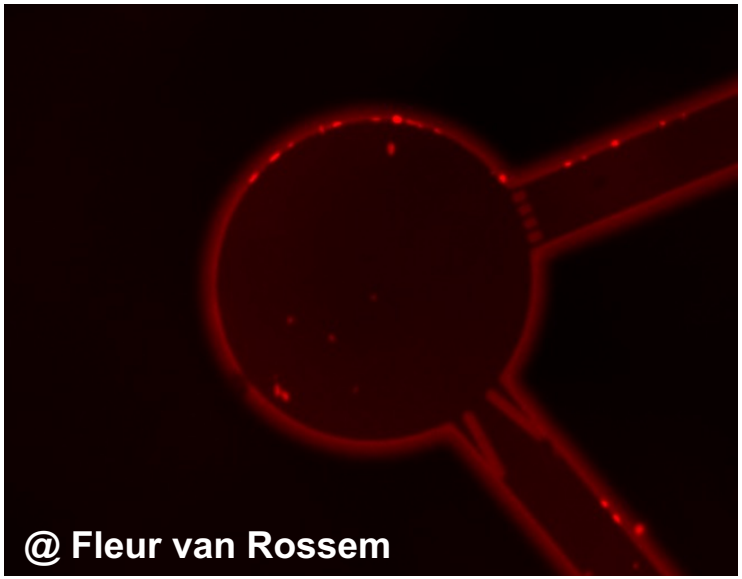


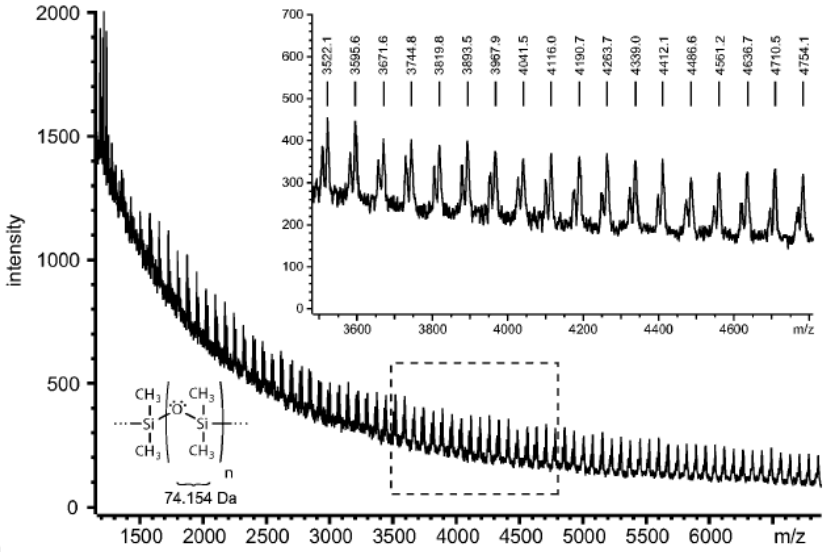
⇨ **Mostly limited to academic prototyping and research**

Materials - PDMS

However…

- **Hydrophobic** material,
- Limited **aspect ratio** in structures, **flexibility** of the material,
- Sensitivity to the **temperature** and to organic solvents,
- **Porous** material ⇨ evaporation of solvents ⇨ concentration changes
- **Gas-permeable**: good or bad news?
- **Adsorption and absorption** of (small) and hydrophobic molecules….
- Material release in solution: **contamination; interactions with cell membranes**





Regehr et al., 2009; Berthier et al. 2012; Beekman et al., 2021

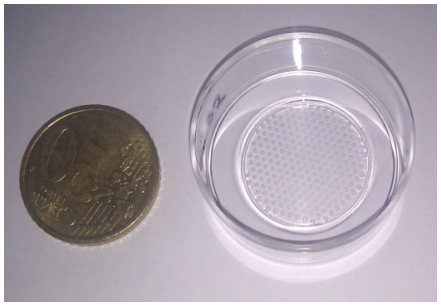
Materials - PS

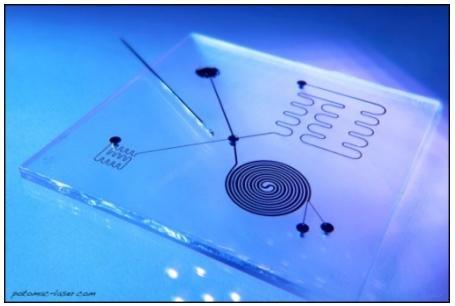
Polystyrene (PS)

- Conventional material for Petri dishes
- Biocompatible, optically transparent, rigid, inert,
- Possible surface functionalization

Techniques for the system fabrication:

- Hot embossing
- Molding of liquid PS

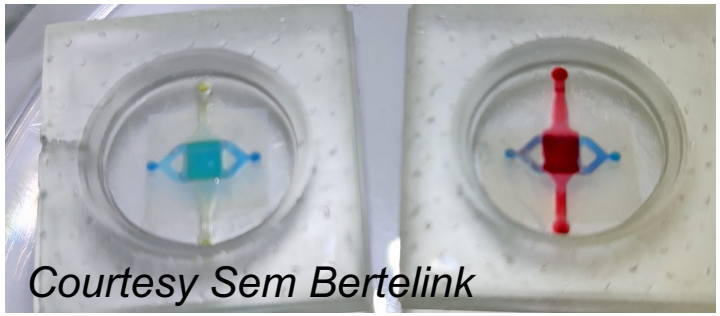




Materials - 3D printed materials

Limitations

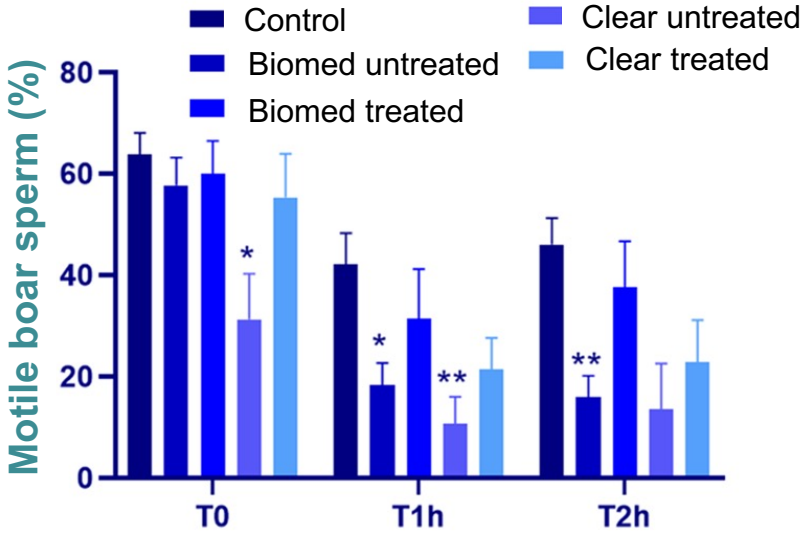
- Resolution of the 3D printed structures, surface roughness
- **Materials**: proprietary composition & biocompatibility

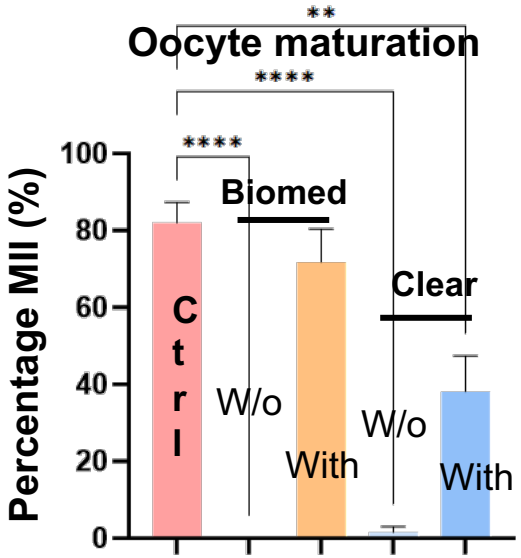


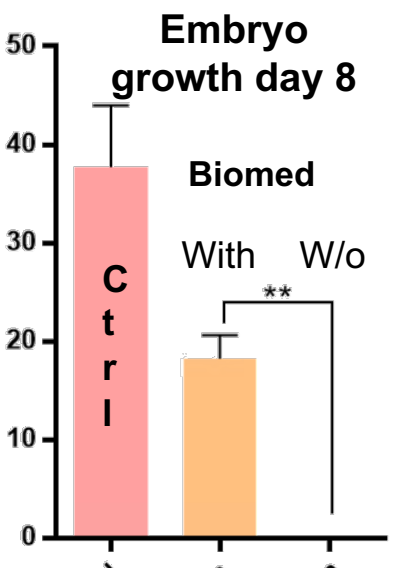
Intended goal

- Oviduct-on-chip model for studying fertilization, chemical toxicity, *in vitro* embryo production

- Material **biocompatibility** with/without treatment for sperm, oocytes, and embryos





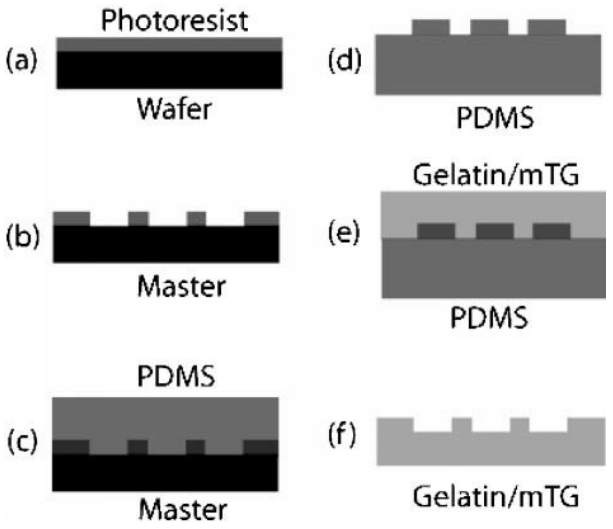


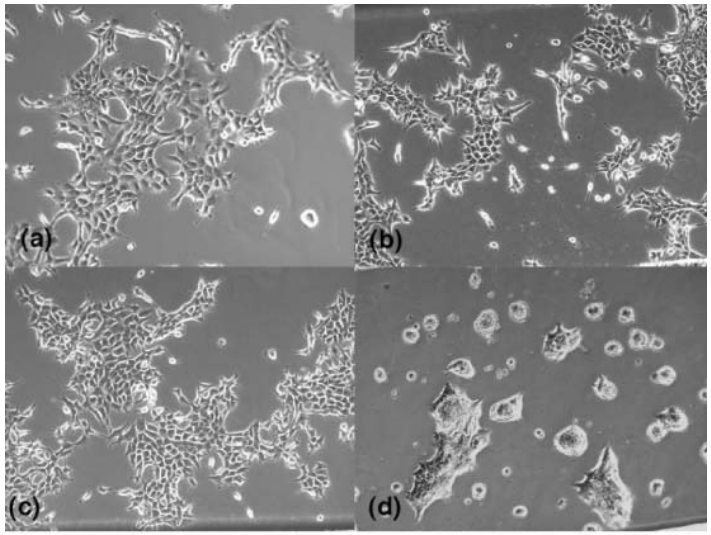
MSc thesis Sem Bertelink; collaboration Prof. Saint-Dizier (INRAE, Tours, FR)

Soft materials – Hydrogel & Gelatin

Soft materials

- Same texture as tissues
- Possibility to alter their mechanical properties
- Highly porous substrates
- Easy molding against, *e.g*, PDMS structures
- Gelatin crosslinked with microbial transglutaminase





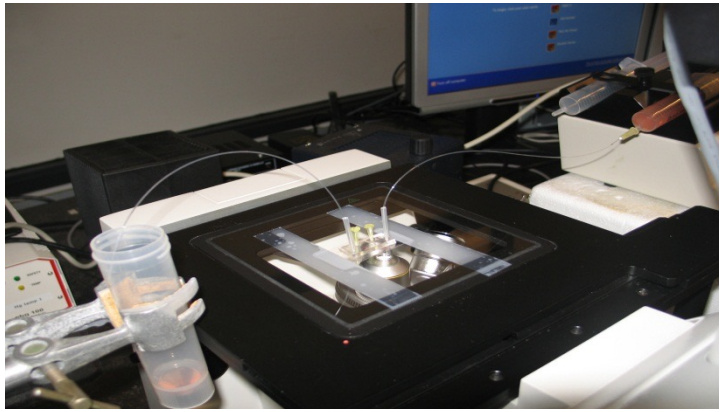
24 hours

- ECM-like substrate
- Cells forming 3D structures like *in vivo*, and not monolayers as observed on culture dishes (hard materials)
- Invasion of the cells in the gelatin matrix

Paguirigan et al., 2006

Pumping liquids

Ideally: continuous perfusion of medium in the microsystem



Issues:

- No shear stress on cells (high flow-rate ⇨ local detachment and death of cells)
- **Homogeneous distribution** of nutrients and gas ⇨ no stagnation point
- **Diffusion-based** transport of nutrients (*in vivo* conditions): **convective** transport at proper flow-rate to maintain the tissue mass.
- No air bubble, T˚C

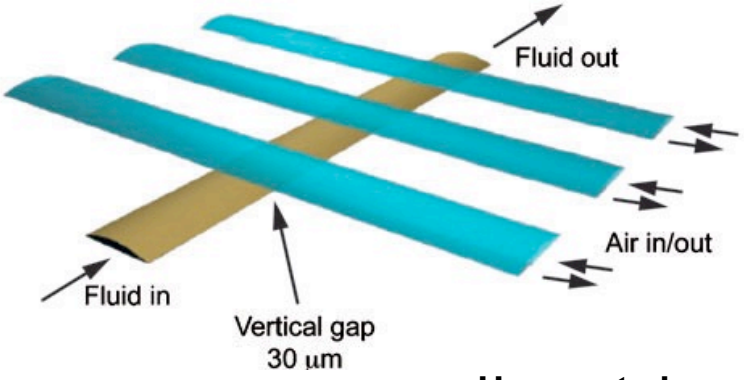
⇨ Mild pumping protocol

⇨ Insertion of a membrane between the flow and the cells

⇨ Purely diffusion-based delivery of fresh medium

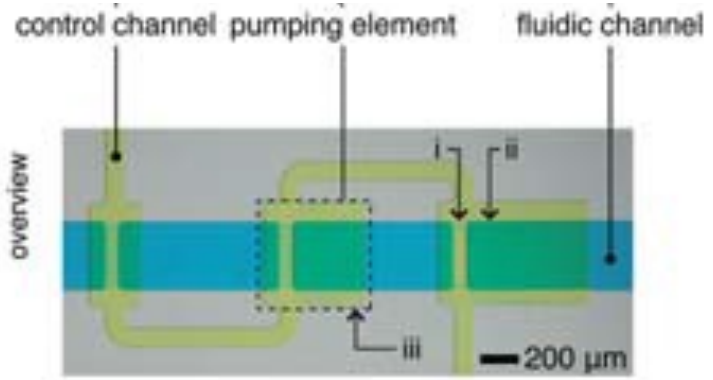
Pumping liquids - Peristaltic pumps

PDMS valves: peristaltic integrated pump



Unger et al.,

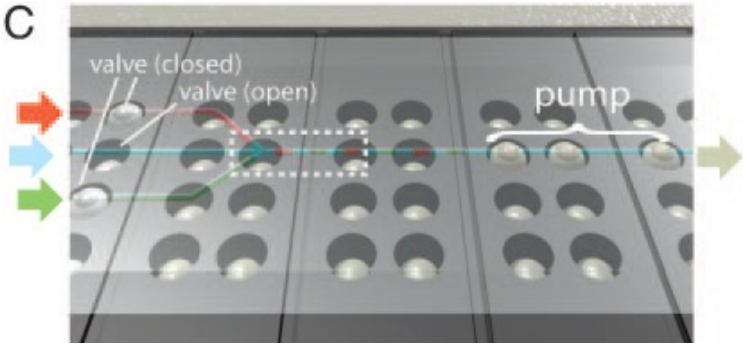
⇨ single-stroke PDMS pump



Lai et al., 2011

Peristaltic integrated pump based on a pin Braille display

- Deformation of a PDMS membrane (140–µm thick) using pins

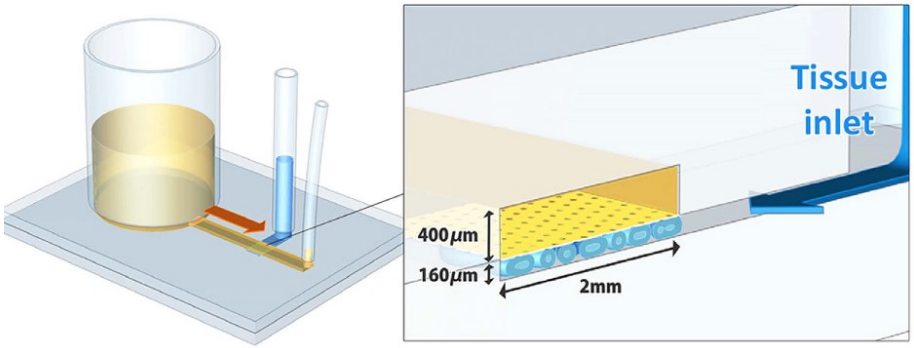


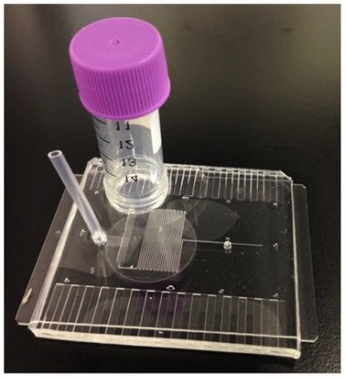
- Very low flow-rate; need for external equipment for valve acuation

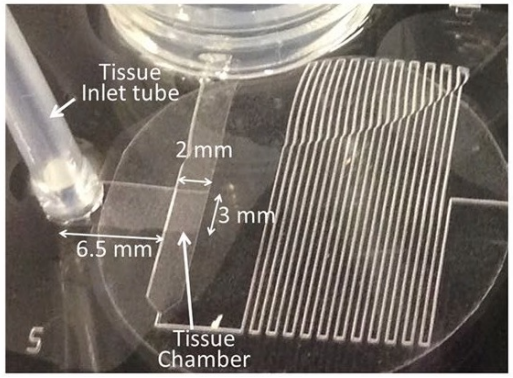
Gu et al., 2004

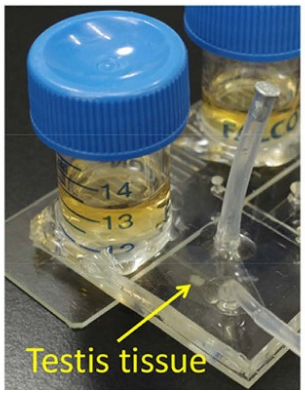
Pumping liquids - Hydrostatic pumps

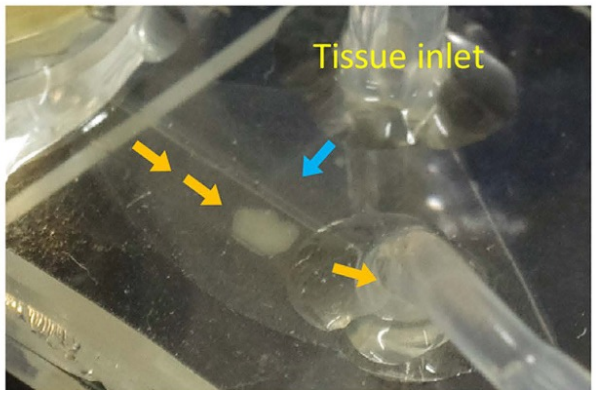
Hydrostatic pumping: using a height difference in liquid for in-incubator pumping, without any capillary connection

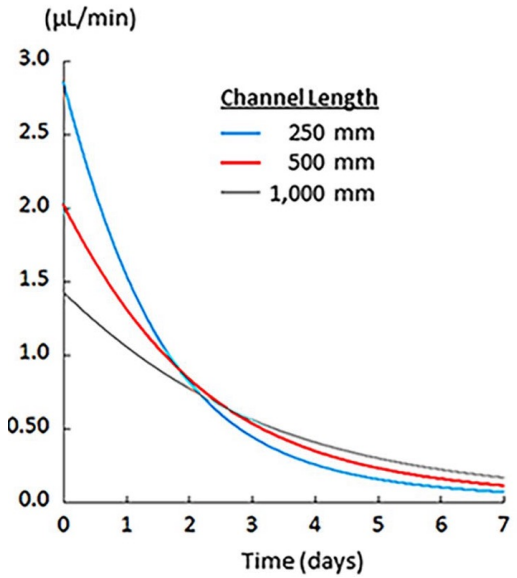










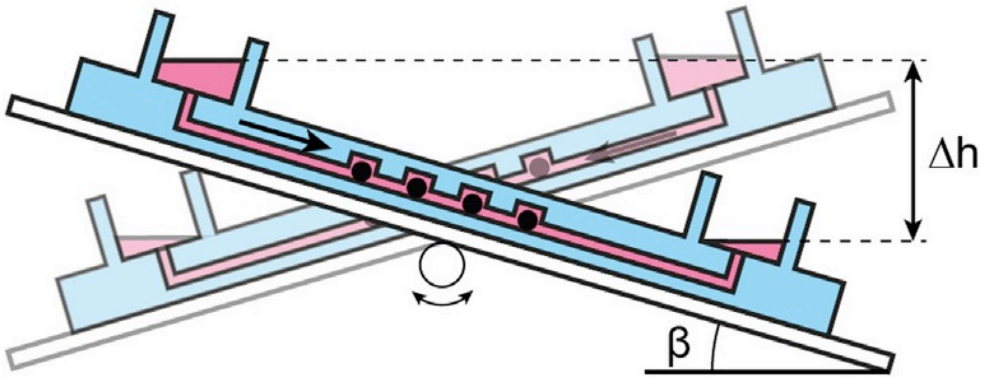


Limitation: no "stable" flow

Komeya et al., Sci. Rep., 2016

Komeya et al., Sci. Rep., 2017

Pumping liquids – Rocking/tilting platform



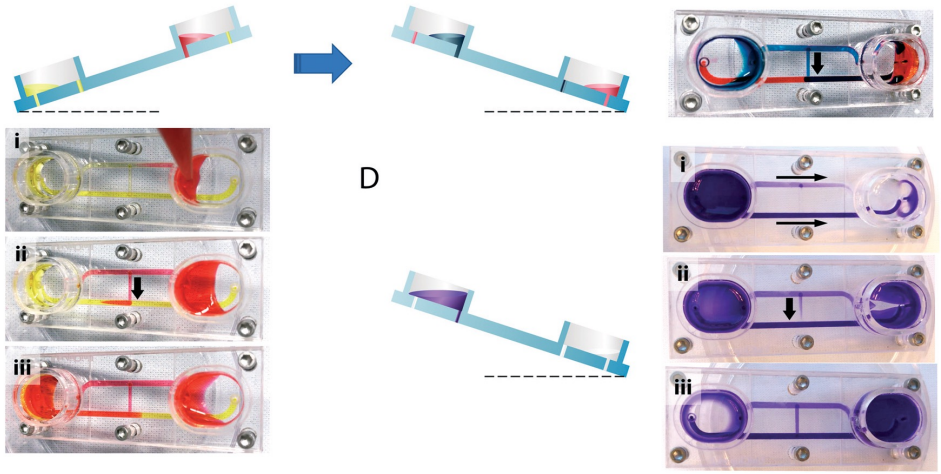
Kim et al., J. Biotech, 2015

Hydrostatic yet "dynamic" pump

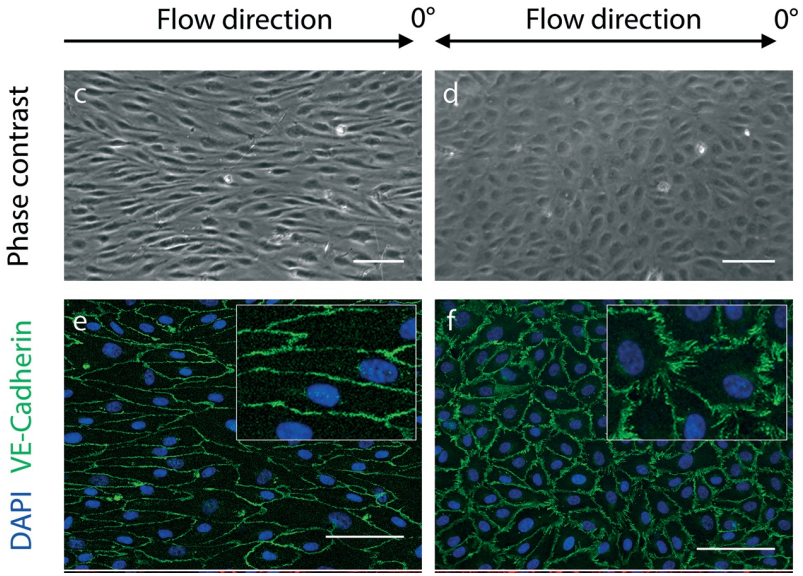
- Use of a tilting or rocking platform
- Continuous flow across the device between two reservoirs; continuous shear on cells
- Instrumentation usable in an incubator
- Operation at a specific "switching" frequency and at a specific angle

Limitations

- **One flow direction**: endothelial cells sensitive to the flow direction

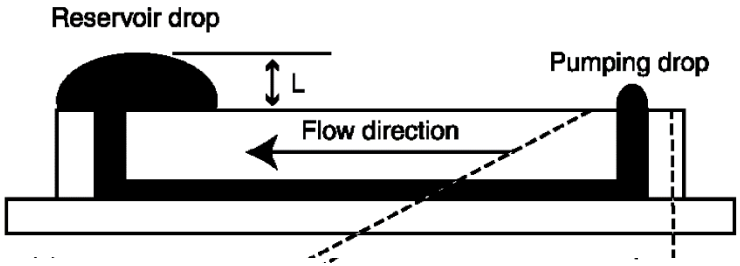


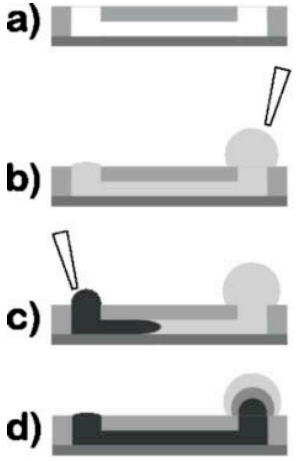
Lab Chip, 2018, **18**, 2563

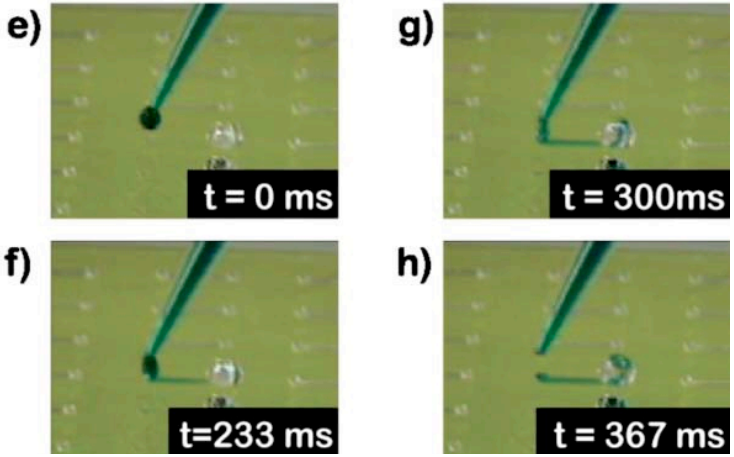


Pumping liquids - Passive pumping

Passive pumping: creation of a flow using surface tension

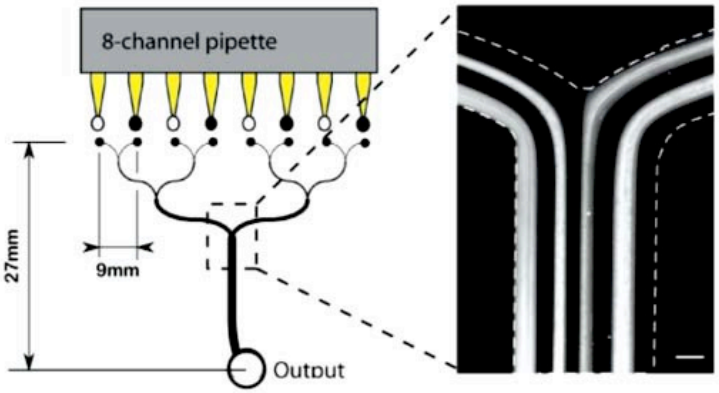






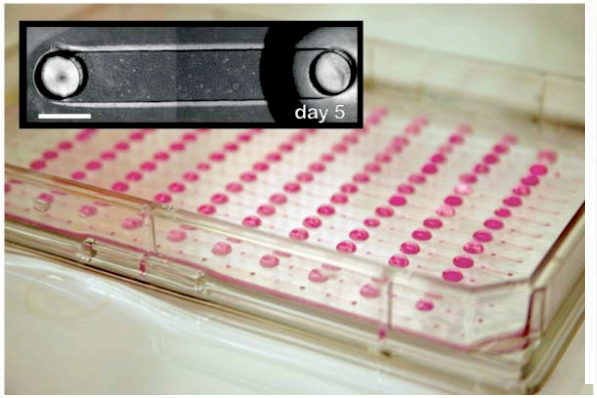
$$\Delta P = 2\gamma/R$$

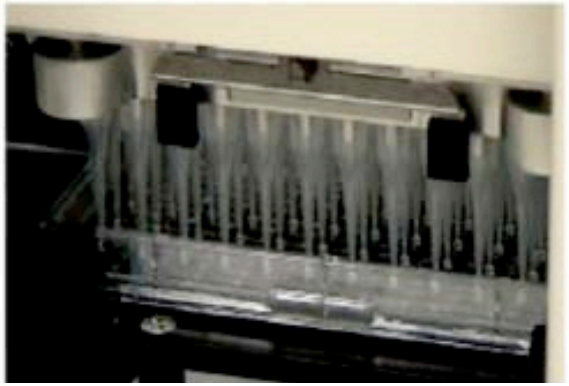
Complex flows



Walker et al., 2002

Multiplexing

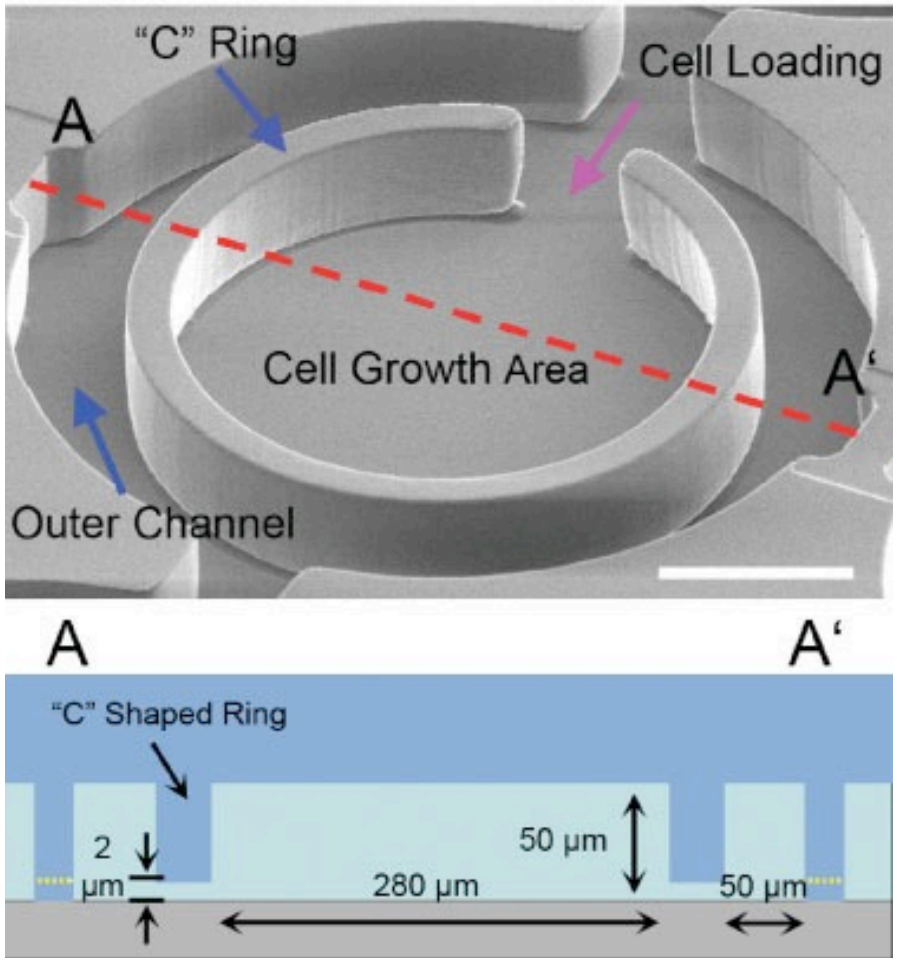


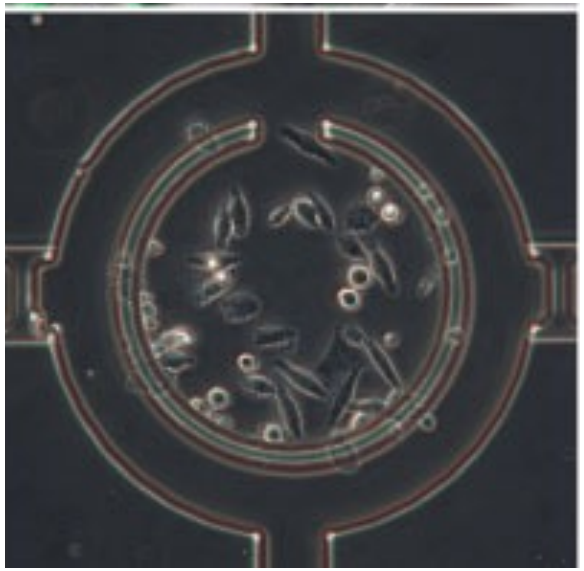


Meyvantsson et al., 2008

Pumping liquids - Diffusion-based delivery

Diffusion-based delivery of medium





Lee et al., 2005

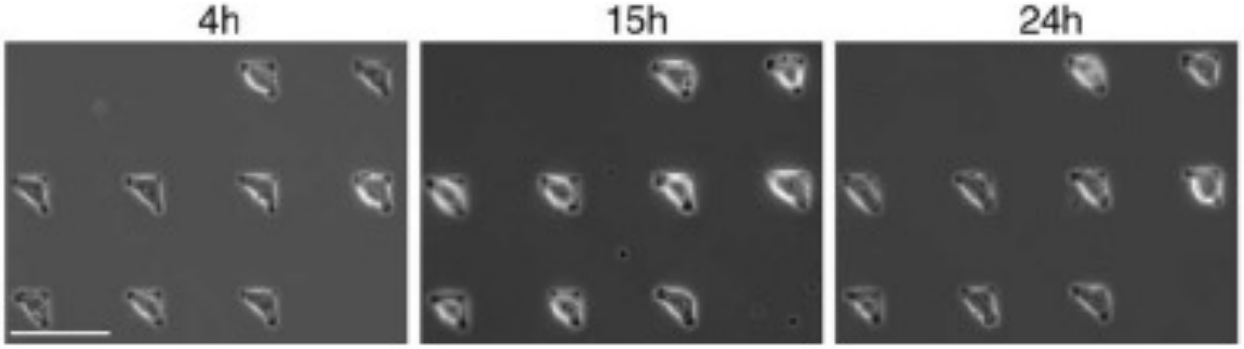
Surface treatment

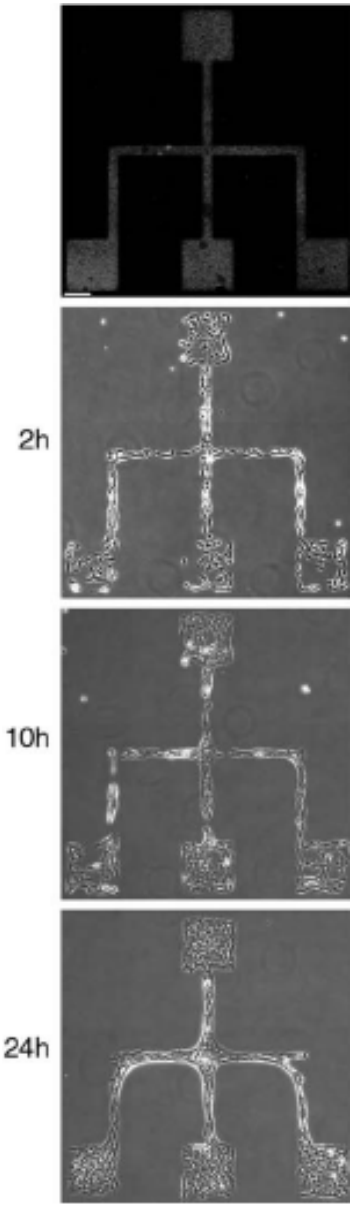
Suspension cells

- **Cell repellent coating** (PEG, BSA) to prevent non specific cell adhesion on the surface

Adherent cells

- **Coating ⇔ controlled cell adhesion**
- Specific coating for their **adhesion**: ECM proteins such as fibronectin, PEI, specific antibodies, polylysine…
- Combination with a **cell-repellent coating** (PEG, BSA)
- Possible **patterned** functionalization for single cell isolation (microcontact printing, spotting)

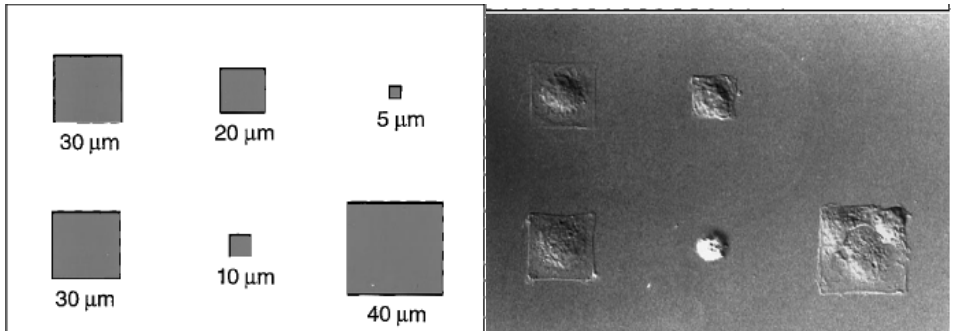


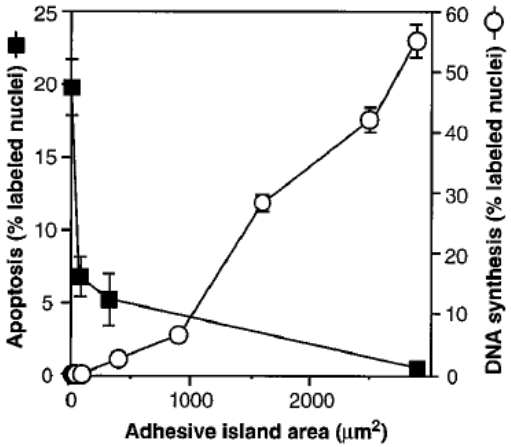


PEG: polyethylene glycol; BSA bovine serum albumine; PEI: polyethylene imine

Surface treatment – Cell differentiation/fate

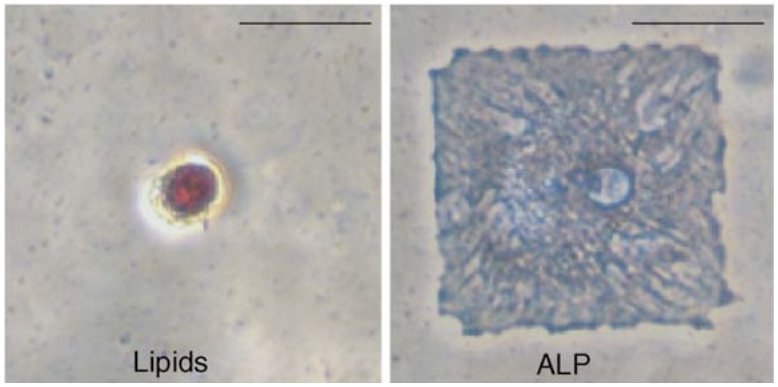
Growth *vs*. apoptosis of cells as a function of their surface area



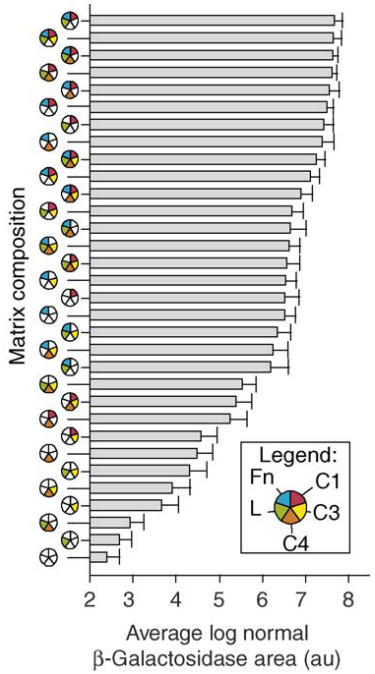


Chen et al., 1997

hMSCs differentiation into adipogenic & osteogenic lineages.



McBeath et al., 2004



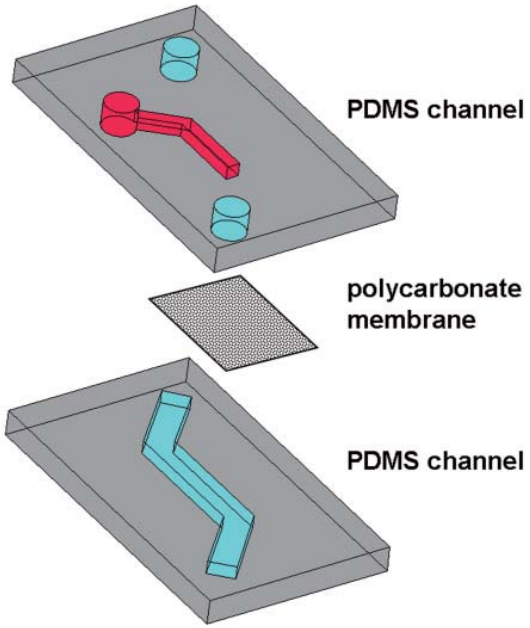
Tuning ES cell differentiation into hepatic lineage by varying the ECM composition.

Flaim et al., 2005

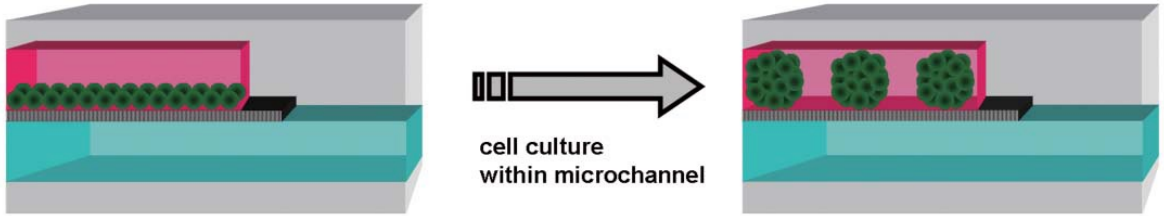
Surface treatment – Spheroid production

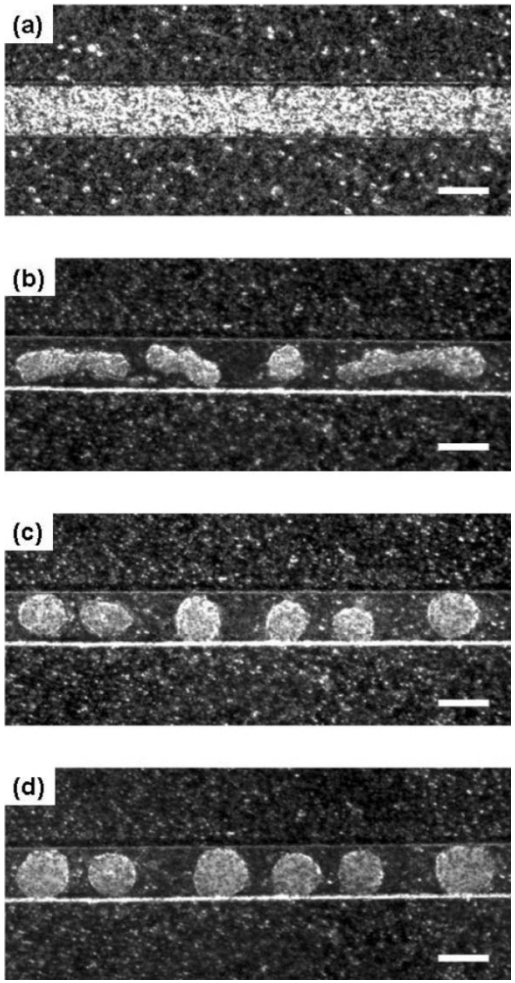
Spontaneous formation of spheroids in microwells and microchannels

- Culture on a cell-repellent substrate ⇨ cell self-assembly into microtissues



- Cell culture on a BSA-coated PC membrane
- Spontaneous aggregation into microtissues





Torisawa et al., 2007

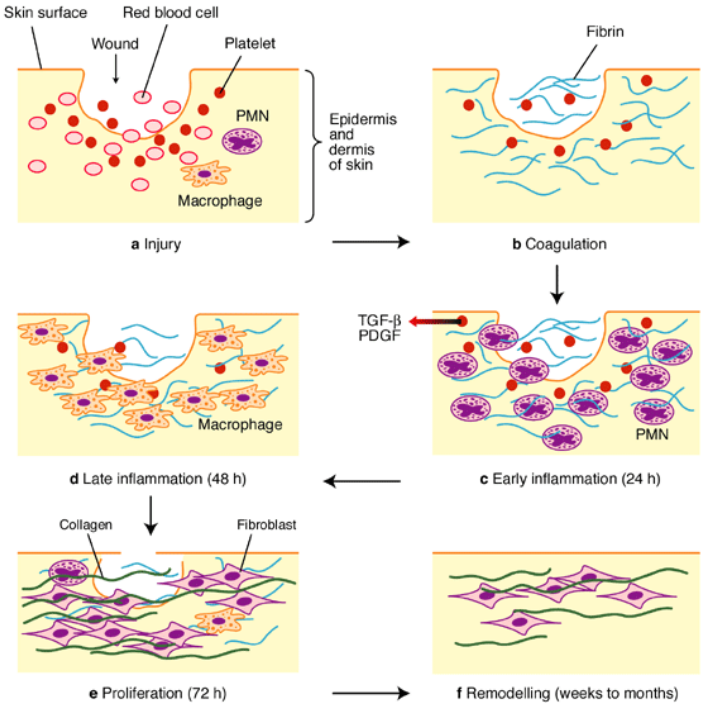
PC: polycarbonate

(Bio)Chemical gradients

Cell microenvironment: not only physical/(bio)chemical cues but also gradients

Gradients

- Regulation of cell behavior: activation of signaling pathways or cell differentiation
- Guide for cell migration (chemotaxis)
- Role in many biological processes (angiogenesis, wound healing, tumorigenesis/ metastasis, development)



Process of wound healing
Successive recruitment of neutrophils (NMPs), macrophages and fibroblasts on the site of injury for wound healing

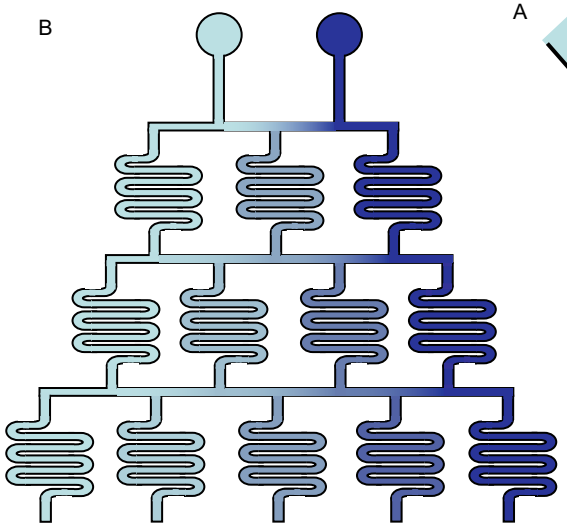
Beanes et al., 2003

(Bio)Chemical gradients

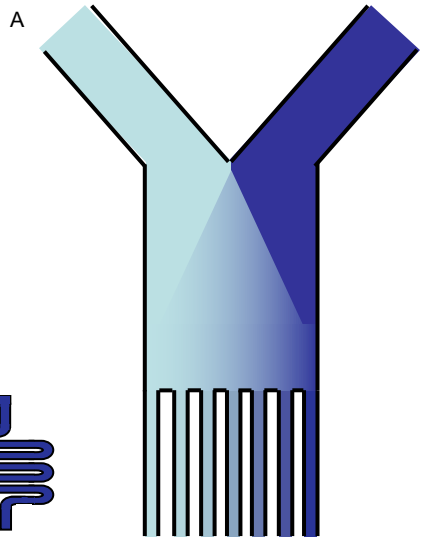
Microfluidics

Straightfoward generation of **gradients** ⇔ predictable and controllable flows:

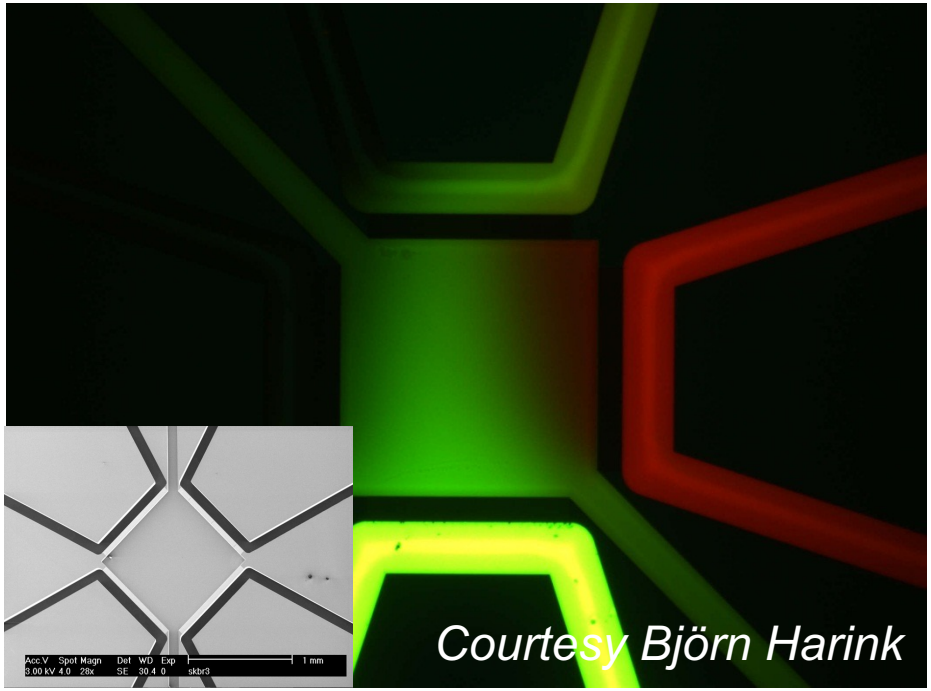
- soluble factors (biochemical cues)
- surface forces (substrate stiffness)
- chemical patterns (molecular functionalization)



Serial mixing of two solutions



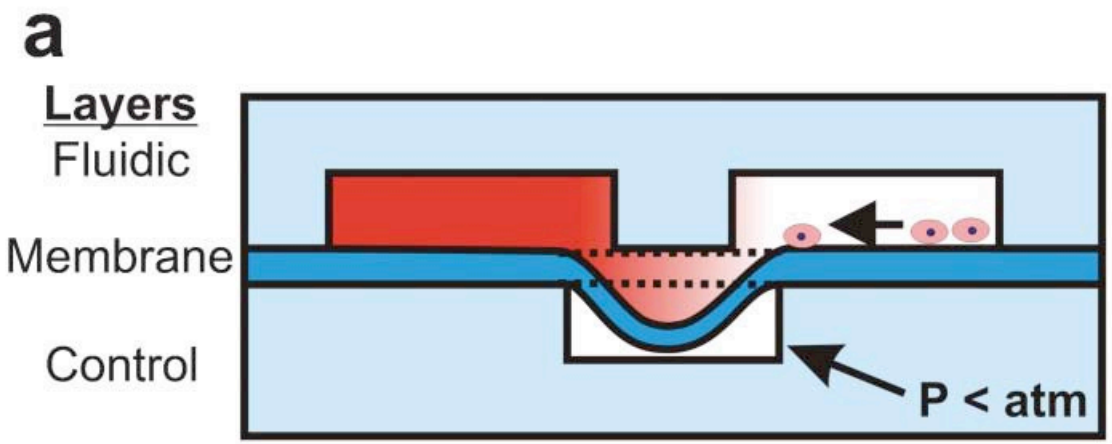
“Mixing” upon diffusion in a flow configuration

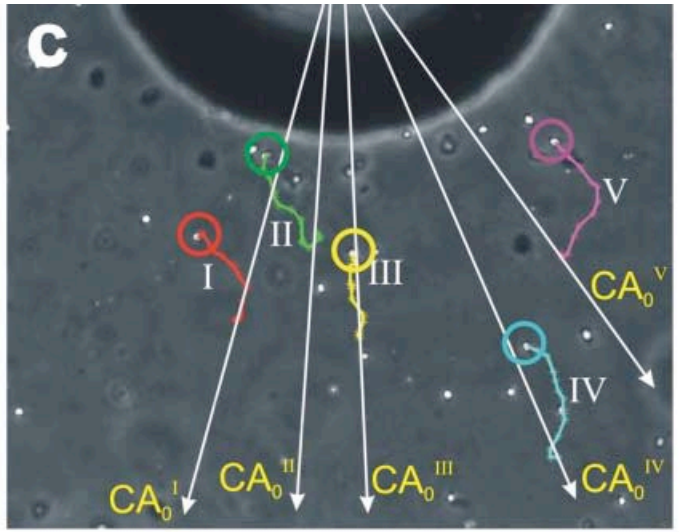


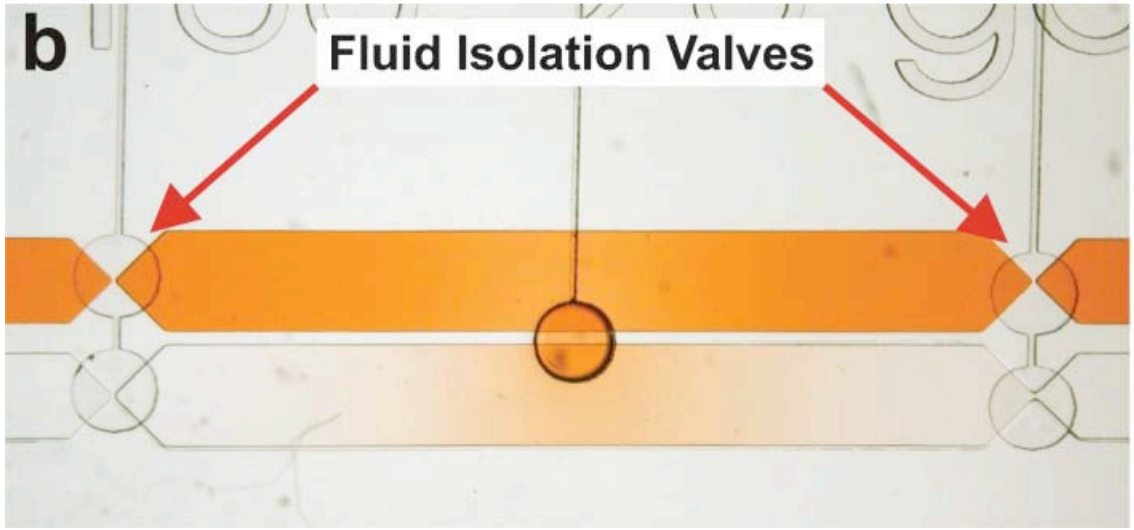
Diffusion-based gradient from a reservoir into a sink via a hydrodynamic barrier

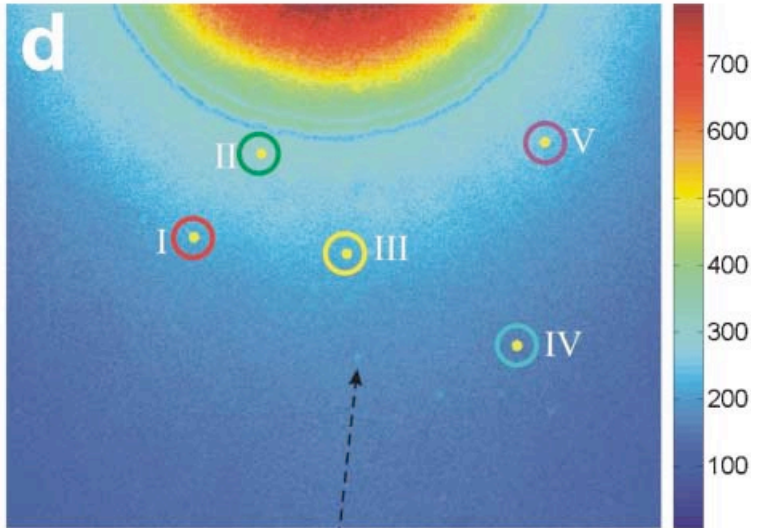
(Bio)Chemical gradients

Chemotaxis study: neutrophil migration towards a source of chemokines



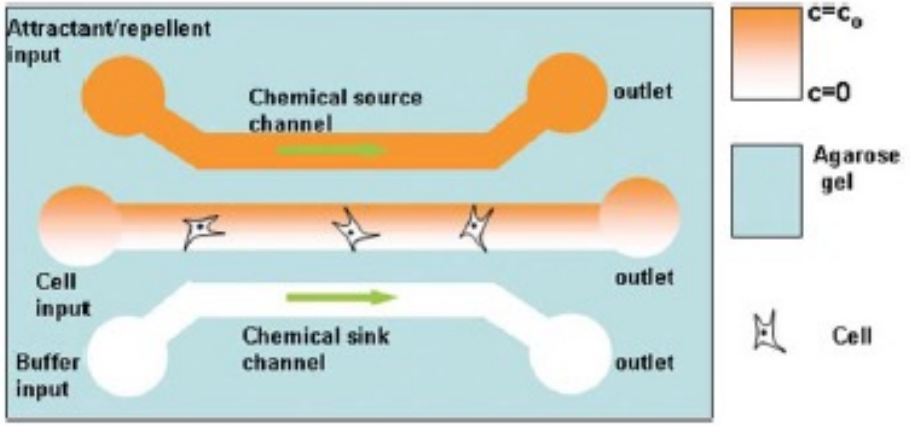




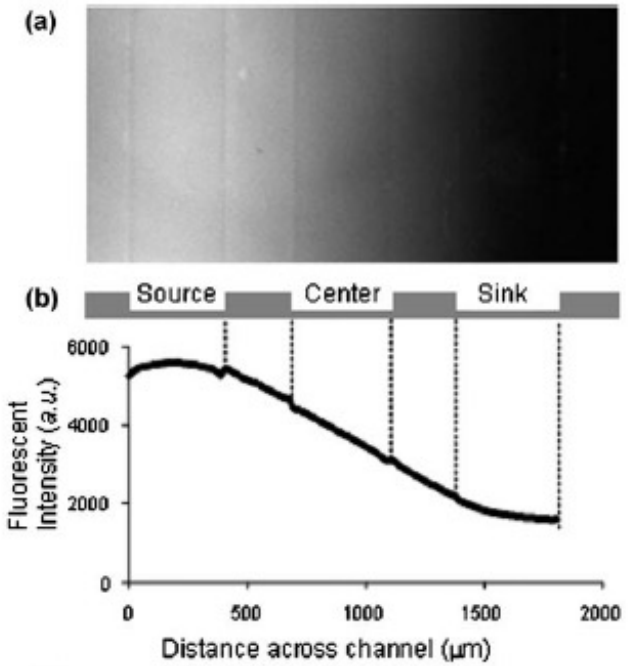


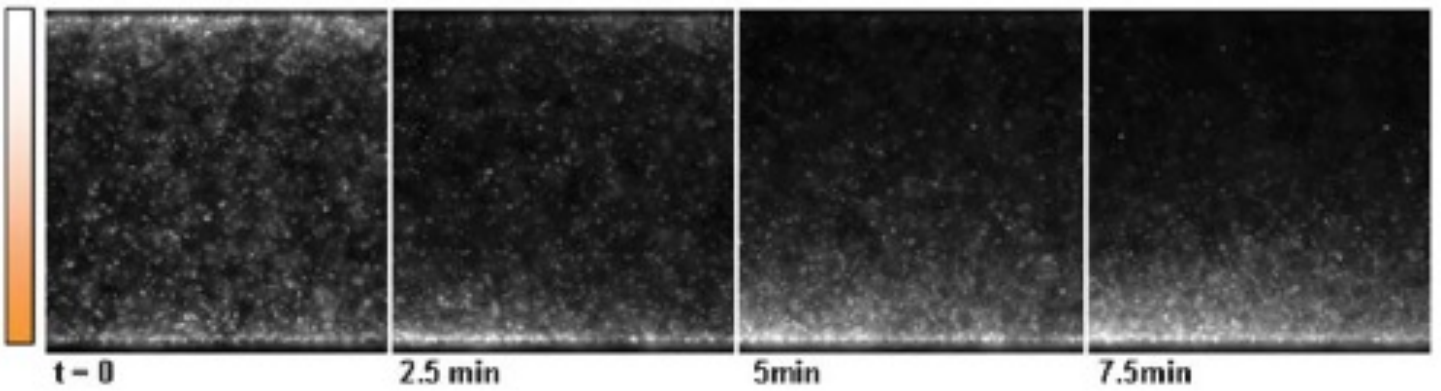
Frevert et al., 2006

(Bio)Chemical gradients



Microdevice made from hydrogel
Diffusion as in aqueous phase

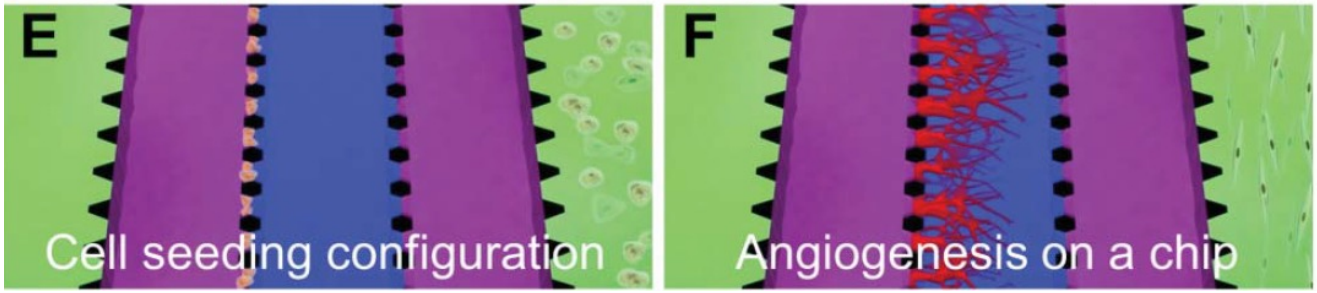




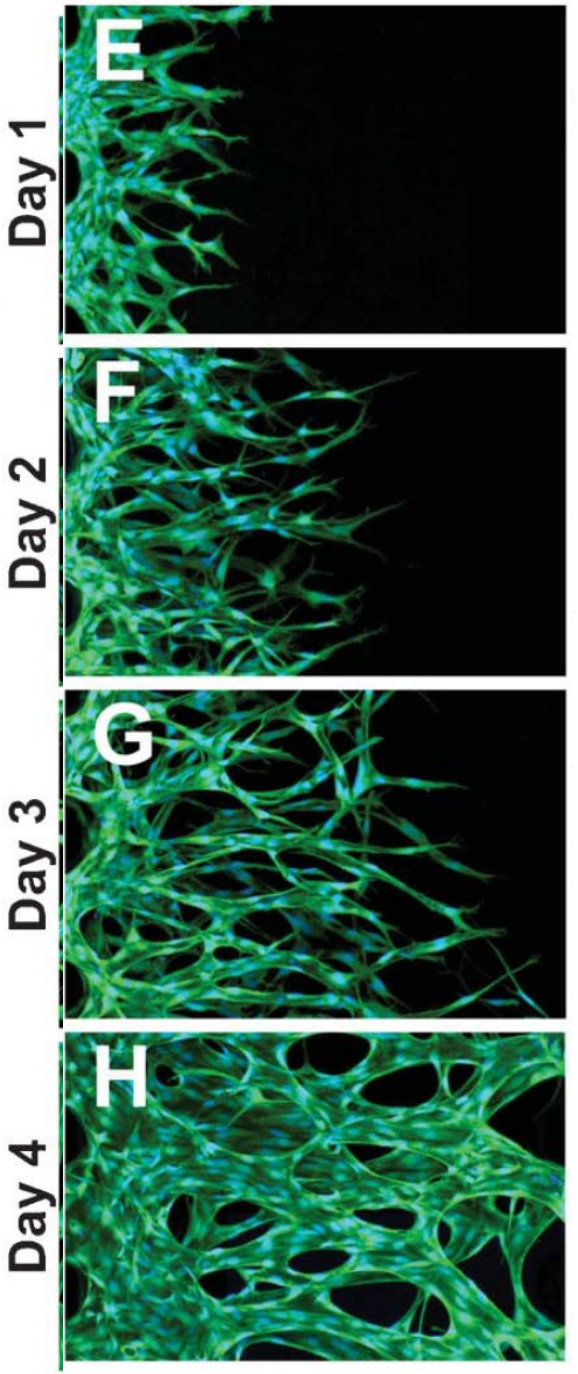
Migration of bacteria (*E. Coli*)

Cheng et al., 2007

(Bio)Chemical gradients Angiogenesis



- Co-culture of endothelial cells and fibroblasts
- **Angiogenesis:** creation of a 3D perfusable vascular network through the migration of endothelial cells into a hydrogel matrix
- Angionenesis proces supported by **growth factors** (VEGF) secreted by stromal cells (here, lung fibroblasts).
- Gradients of growth factors that are secreted by fibroblasts created across a tissue/hydrogel compartment.



Kim et al., Lab chip, 2013; DOI: 10.1039/c3lc41320a

Conclusion – Take home message

Microfluidics

- New tool for exciting and unprecedented research in cell biology
- Possibility to recapitulate the natural microenvironnment of cells

Care to be taken to **design microsystems** depending on the application

- Proper choice of the material
- Need for proper surface chemistry?
- Methodology for pumping fluids