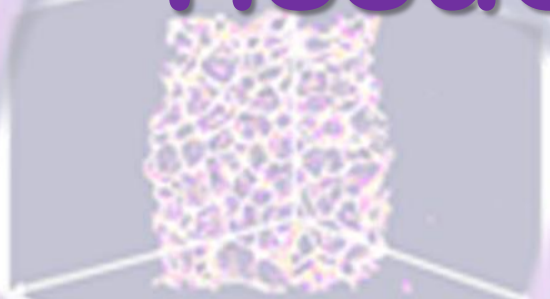
Lesson 5 – 15/12/2015

Bioreactors for Tissue Engineering



Serena Giusti
serena.giusti@centropiaggio.unipi.it

PSWD for zip files: Bioreactors2014

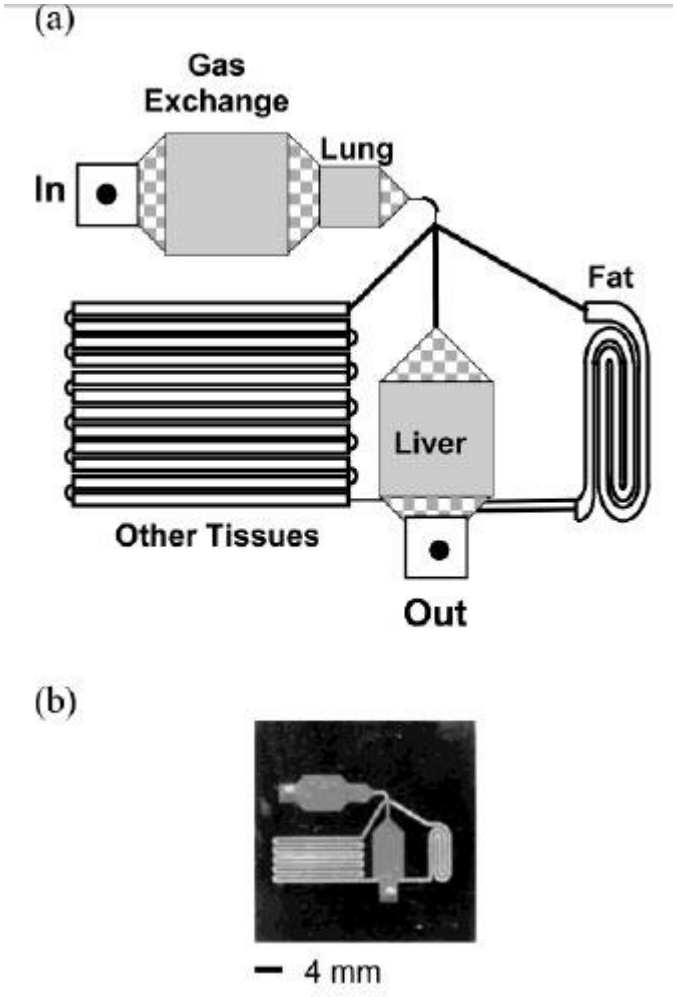
Connected Cell Culture

Coltivazione in connessione con altri sistemi/tessuti

Sono sistemi atti alla valutazione dell'interazione fra i diversi tessuti.

Sono utilizzati in:

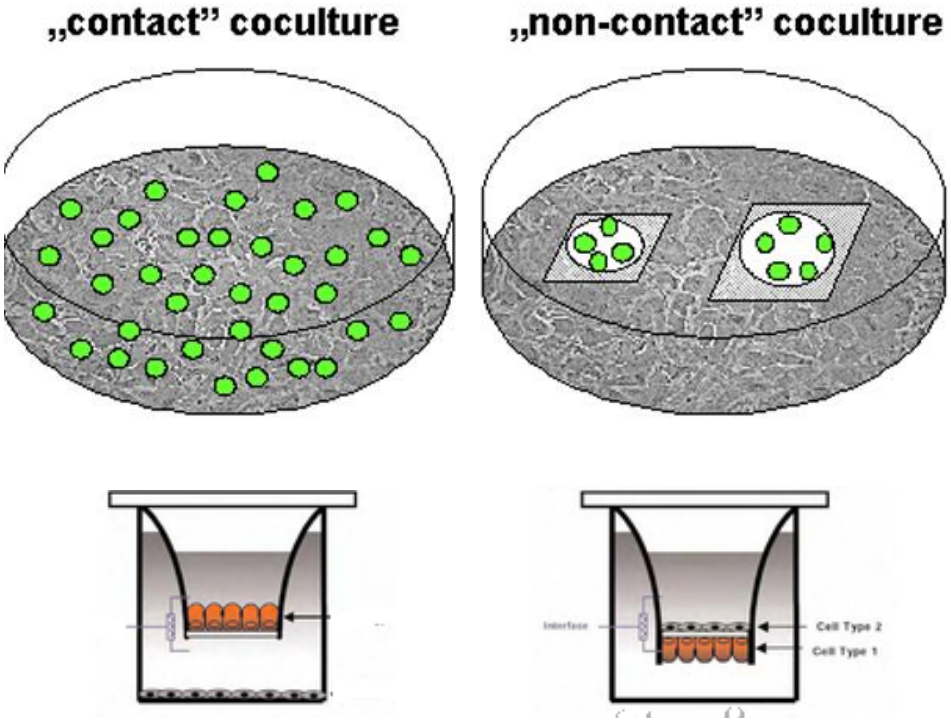
1. Studi farmacologici di attivazione o disattivazione di molecole da parte di tessuti (generalmente fegato e tessuti target)
2. Studi di Cross-Talking cellulare
3. Ogni qualvolta si debba investigare l'interazione fra diversi tessuti, senza che questi siano coltivati in diretto contatto l'uno con l'altro (co-cultura)



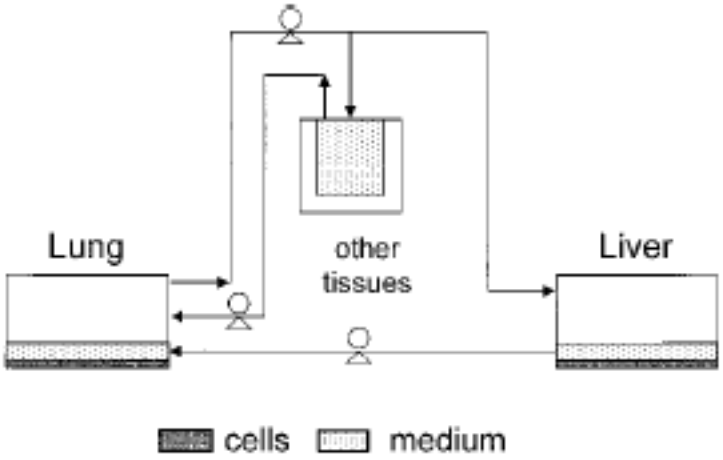
I bioreattori multi compartimentali o interconnessi sono diversi dalle co-culture!!

Co-cultura ≠ Riutilizzo del terreno ≠ Coltura connessa

Connected Cell Culture vs Co-coltures



Co-colture
≠
Conditioned media



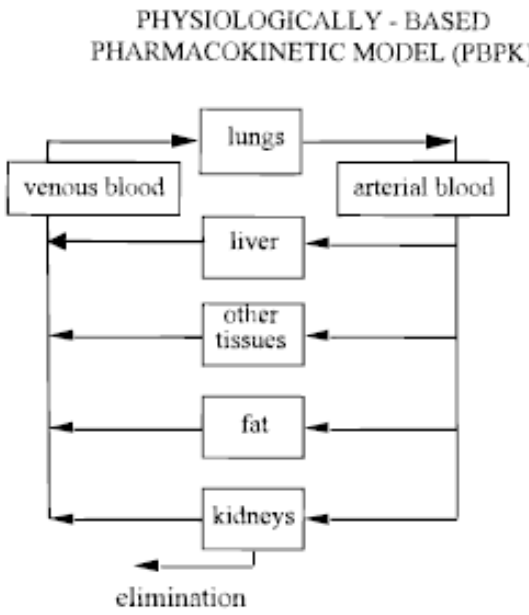
Connected colture

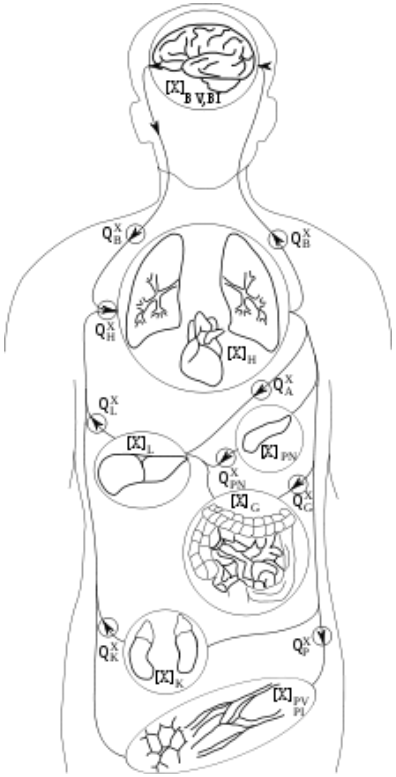
Connected Cell Coltures – PBPK model

- The **PBPK (Physiologically based pharmacokinetic)** is mathematical modeling technique for predicting the Absorption, Distribution, Metabolism and Excretion (ADME) of synthetic or natural chemical substances in humans and other animal species
- The PBPK model divides the body into **compartments** representing **organs**, integrating:
  - kinetic
  - thermodynamic
  - anatomical parameters

$$\frac{dQ_i}{dt} = F_i(C_{art} - \frac{Q_i}{P_i V_i})$$

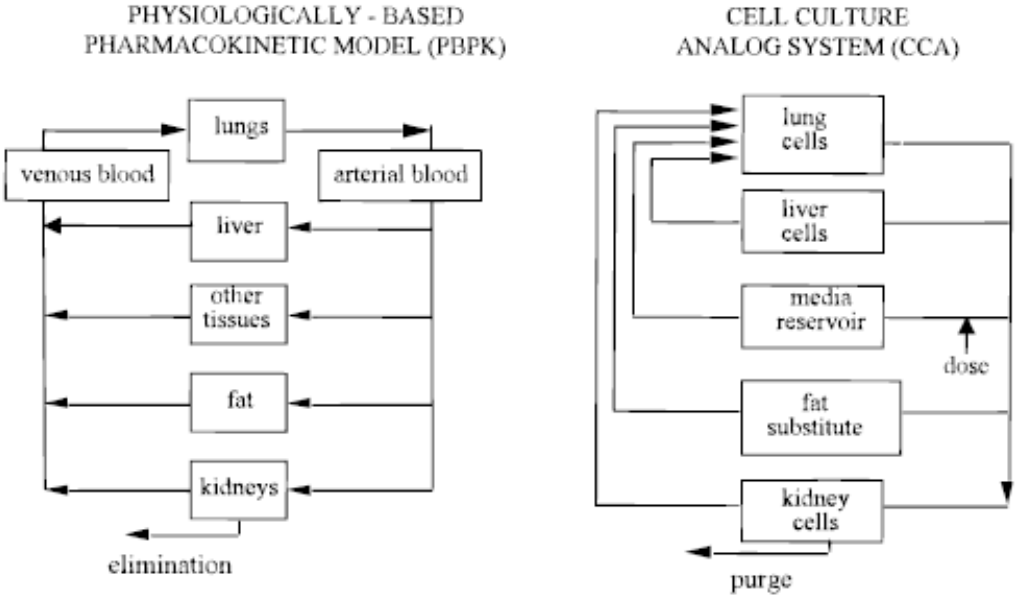
where
F_i is blood flow,
C_{art} incoming arterial blood concentration,
P_i the tissue over blood partition coefficient
V_i the volume of compartment *i*.



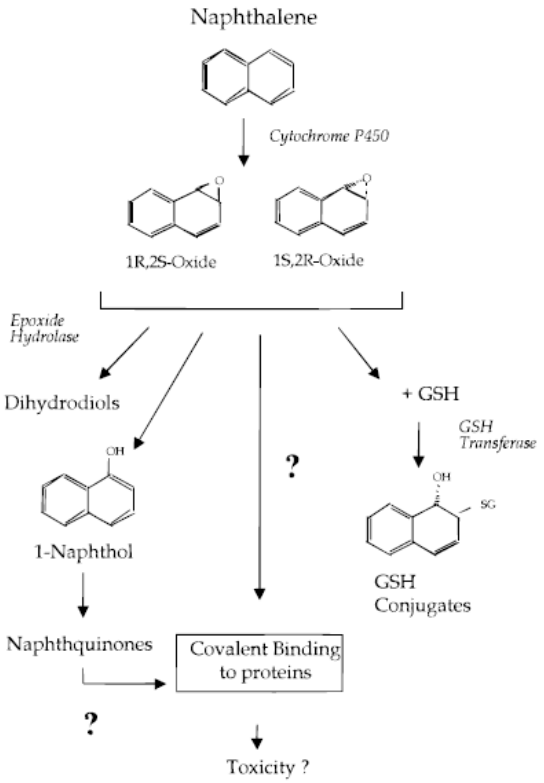


Connected Cell Coltures – PBPK model

- The *cell culture analogue* reactor (CCA) is a physical replica of the PBPK; where the PBPK specifies an organ or tissue compartment, the bioreactor contains compartments with a corresponding cell type.
- A cell culture analogue of a PBPK can be constructed replacing each PBPK compartment with a biological system that contains the key reactions corresponding to the PBPK kinetic and thermodynamic equations.
- The device is a **continuous**, **dynamic system** composed of multiple cell types that interact through a common circulating cell culture medium.



Case study: naphthalene

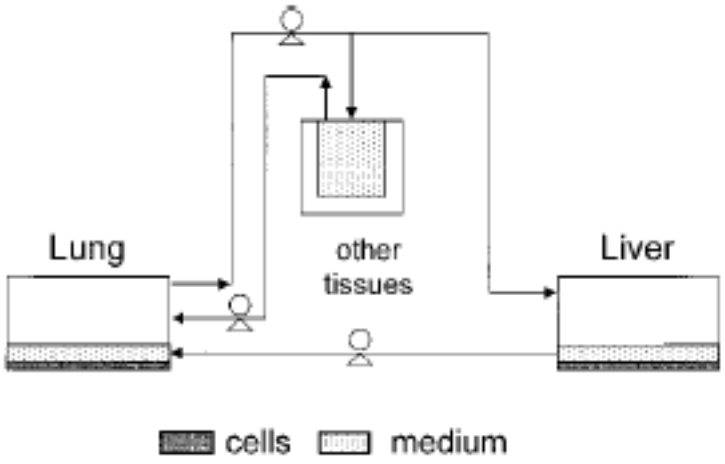


Naphthalene has been selected as a model compound for proof-of concept studies:
naphthalene metabolism is representative of a broad range of nonnutritive polycyclic aromatic hydrocarbons.

Naphthalene is metabolized in the body by the cytochrome P450 monooxygenase system (CYP450).*The primary site of naphthalene* metabolism is the liver, where it is metabolized into naphthalene oxide enantiomers.
The primary target of naphthalene is the non-ciliated bronchiar endothelial cells.

Key points

- In the CCA is used only a single cell type in each “organ”: L2 cells in the lung compartment and H4IIE cells in the liver compartment
- the “organ” compartments contain the key enzymes of the PBPK model.
- The CCA has advantages over other in vitro systems, thus to allow quantitative measurements



- **A 300 mg/kg** dose of naphthalene (dissolved in methanol)

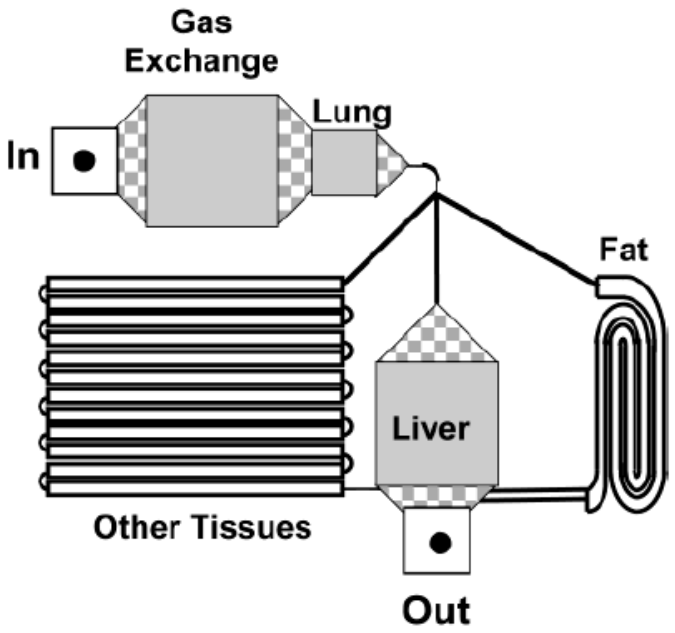
Results:

- Depletion of intracellular gluathione in the L2 cells 6 h after dosing.
- **Depletion in liver cell glutathione** is shown after 4h from dosing, but not at 6h
- *Controls for the experiments* were flasks of the same cell types prepared in the same way as cells in the reactor but kept in an incubator during the reactor run, and different CCA configurations.

The «micro» CCA-PBPK

- A microscale CCA (μ*CCA)*, *also called* "**animal-on-a-chip**", may be constructed to better mimic the appropriate physiological scale (e.g., characteristic dimensions of tens of microns).

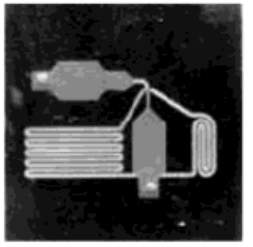
compartment	calculated residence time (s)	measured residence time (s)
lung	2	2
liver	28	38 ± 10
fat	134	120 ± 12
other-tissue	207	200 ± 20



The culture medium first entering the system through the inlet passes through to the "lung", after which;

- 9% to the fat chamber
- 25% to the liver chamber
- 66% "other tissue" chambers

(b)



The «micro» CCA-PBPK

Cell cultures:

The lung and liver chambers were then coated with Matrigel at 100 µ*g/cm2* density:

- 40 µ*L of L2 at 2 X10^5 cells/mL were plated into lung* chamber (8000 cells)
- 40 µ*L of HepG2/C3A or H4IIE* at 1X10^6 were seeded into liver chamber (40000 cells) **5 hep : 1 Lung**

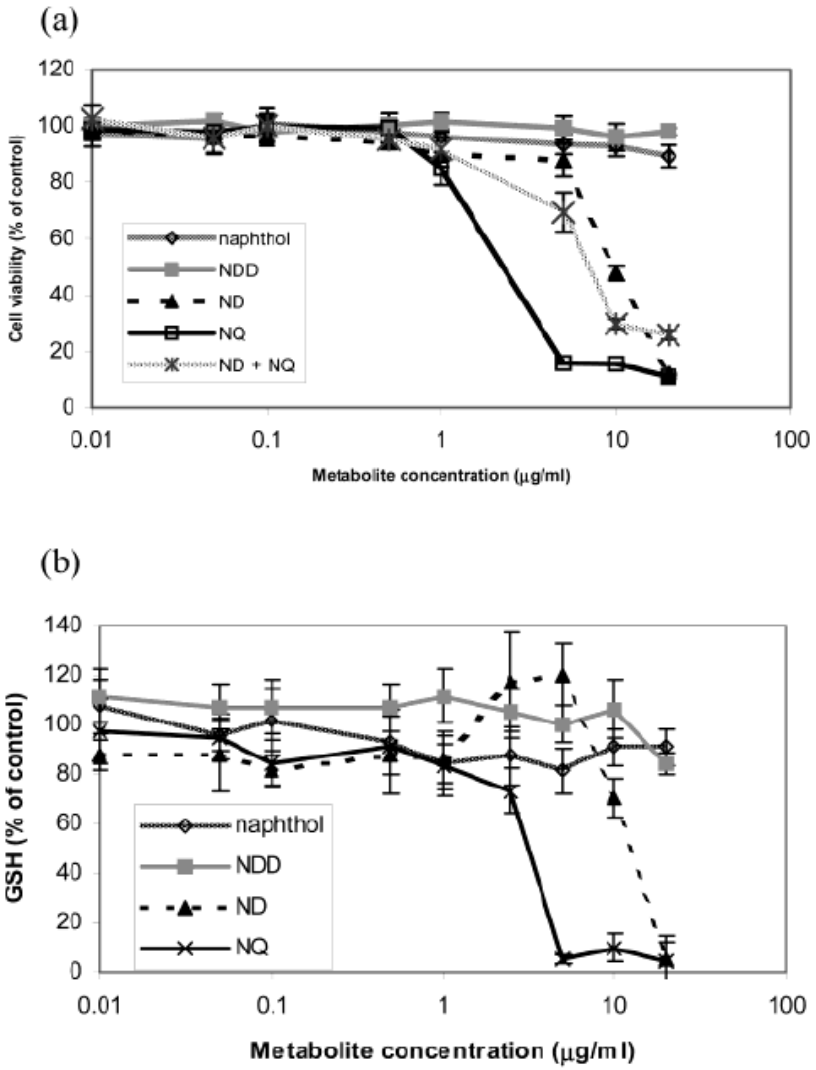
Flow at 2 µ*L/min.*

NB. In the «fat» and «other tissues» compartments there were NO CELLS

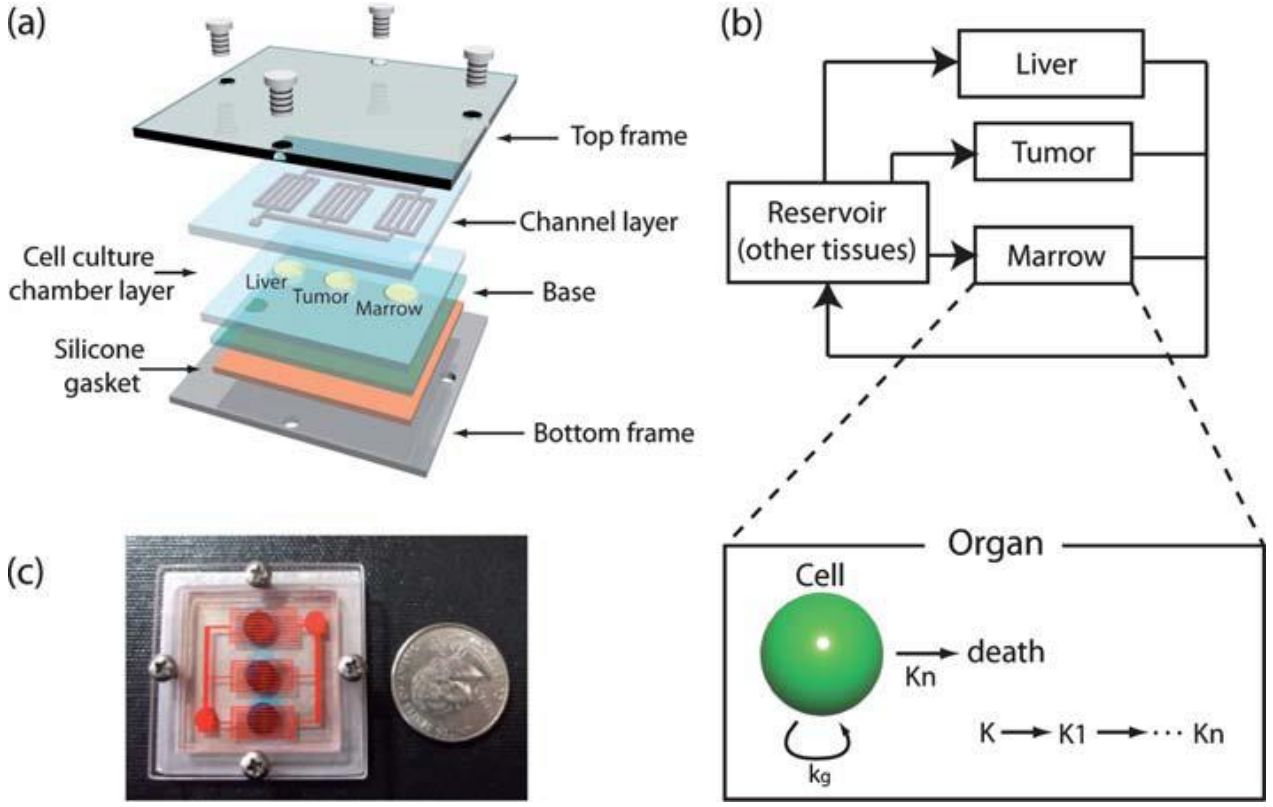
The “fat” compartment is added to a previous three chamber íCCA system to better mimic the fluid distribution in rapidly and slowly perfused organs and more accurately predict the response of animals to chemicals.

Results µCCA

- naphthalene diol and naphthoquinone significantly decreased cell viability
- Calcein stain revealed that naphthoquinone drastically changed the morphology of L2 cells
- *Unlike L2 cells,* the morphology of C3A did not change upon being exposed to naphthoquinone
- GSH in both cell lines, L2 and C3A, decreased with increasing concentration of naphthoquinone
- After 6 h experiment, there was no intracellular GSH depletion in L2 observed in the L2-blank and L2-L2 combinations

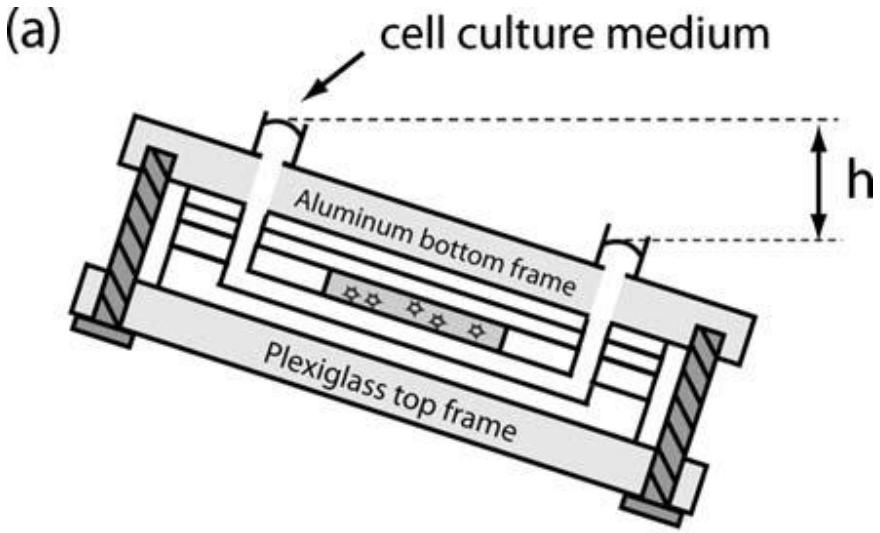


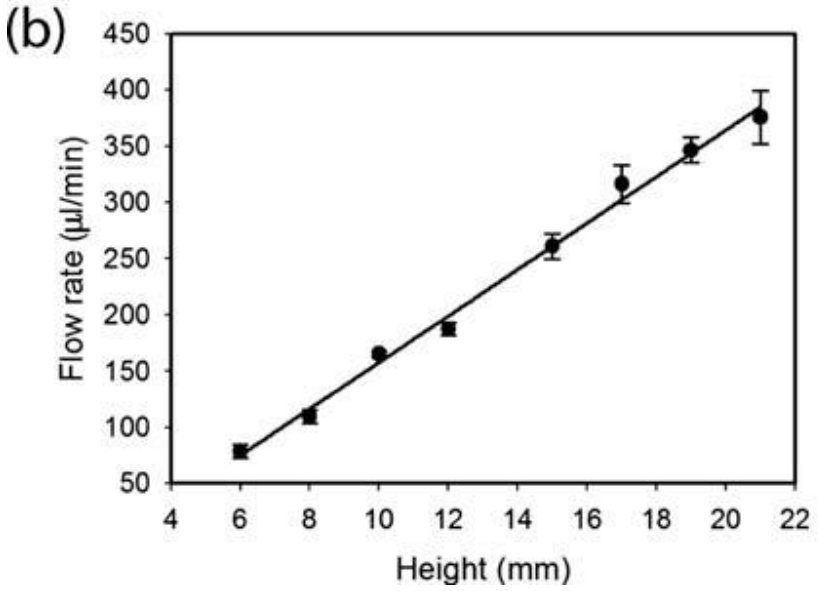
A new application for μCCA



(a) A schematic of device components. A fluidic channel layer and a cell culture chamber layer are superimposed and sealed by top and bottom frames. A silicone gasket and a polycarbonate base are inserted for sealing. (b) A corresponding PBPK model, with the liver, tumor and marrow compartment. Below is a PD model for cell death in each compartment. Although not drawn explicitly, a PD model for each compartment exists separately, and the 'organ' can be the liver, tumor or marrow. (c) A picture of the assembled device. A red dye was used for visualization of channels, and a blue dye was mixed with alginate.

A new application for μCCA





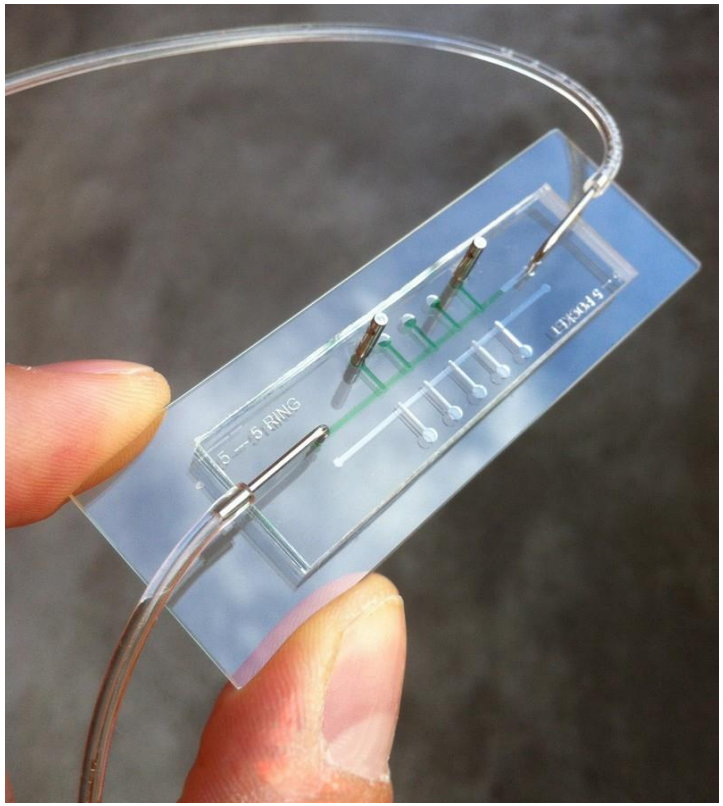
Although microfluidic systems have a great potential in enhancing the drug development process, actual applications of microfluidic systems in medical or life science area have been limited.

- current microfluidic devices require specialized skills for fabrication and operation, which makes it difficult to be used by non-experts.
- microfluidic cell cultures need more indepth study, such as maintenance of sterility, formation of air bubbles, the effect of shear stress on cells, and the «edge-effect»

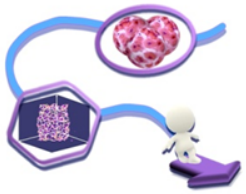
The use of gravity-induced flow eliminates the need for a pump, and prevents formation of air bubbles.

Milli VS Micro scaled systems

Micro-fabricated micro-fluidic cell culture systems are highly cited, but remain a **niche research tool** with **several intrinsic limitations**



- **Cell culture surface** is ~ **0.5-0.8 mm²** and **seeded with few thousand cells**
- The **surface/volume** ratio is **extremely high** giving rise to
  - **High wall shear stresses** having **detrimental effects on many cell types** (e.g. hepatocytes)
  - **Edge-effect** with **peripheral cells** exhibiting **increased cytoskeletal tension** and **altered viability/functionality** than central ones
  - **Air bubbles** causing **fluid pattern alterations**
- **PDMS** to **enhance oxygen transport**
  - **Adsorbe small hydrophobic molecules** (nutrient/ligand depletion, exp. artefacts)
- **Very difficult** to **handle**, **assemble** and **use**



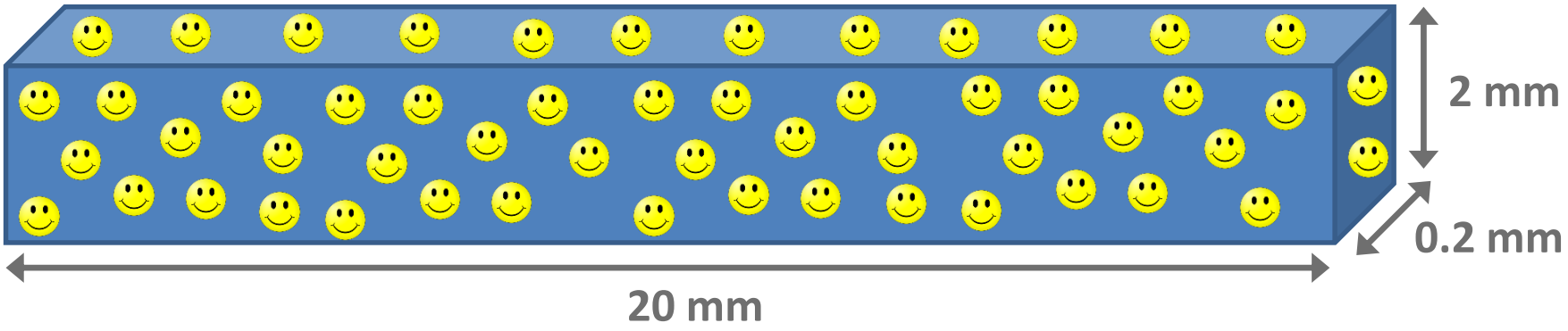
Milli VS Micro scaled systems

Micro-fluidic systems also **require** the **translation of conventional experimental methods and protocols** established at the **milli-scale** using **standard cell culture supports** (e.g. 24 MW plate).

Micro-Fluidics	Milli-Fluidics
Low shear by reduction of flow rate	**High flow rates with low shear**
Low nutrient turnover	**High nutrient turnover**
High surface to volume ratio	**Low surface to volume ratio**
Fiddly to assemble	**Easy to assemble**
Presence of air bubbles	**No air bubbles**
Low fluid volumes, saving on reagents	Higher volumes of media and reagents
Easy quantification of cell products	Cell products may be harder to quantify

Milli VS Micro scaled systems

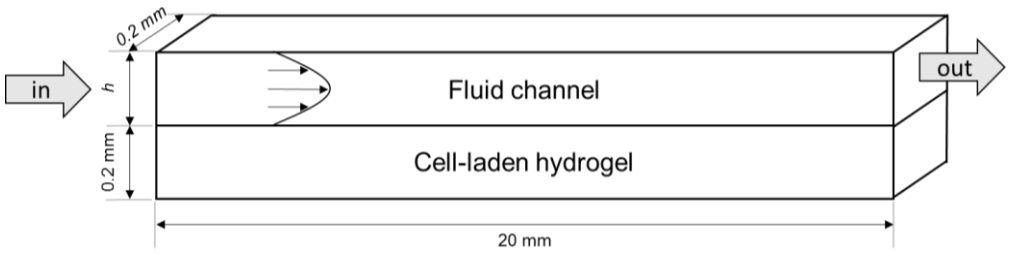
Surface of hepatocyte-laden construct in **equilibrium** with **maximum** c_{O_2} (i.e. **0.21 mM**)



Not implementable in real fluidic systems as it requires **very high medium flow rates** that **damage cells** due to **high shear stresses**

Milli VS Micro scaled systems

Simple parallelepiped fluidic channel



- ✓ **PDMS milli-molded culture chamber**
- ✓ **Shape and dimensions similar to** those of a **24 MW plate well**
- ✓ **Suited for** implementing **multi-organ in-vitro models** to study **organ cross-talk** through **inter-connected cell cultures**

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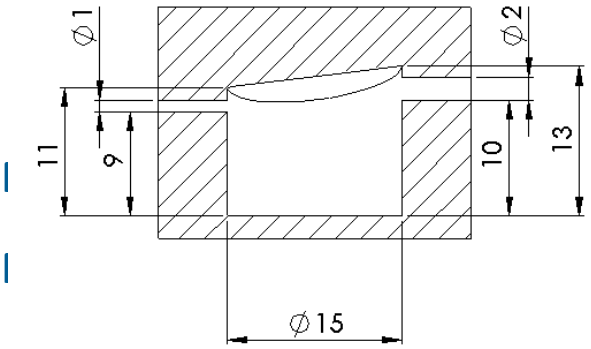

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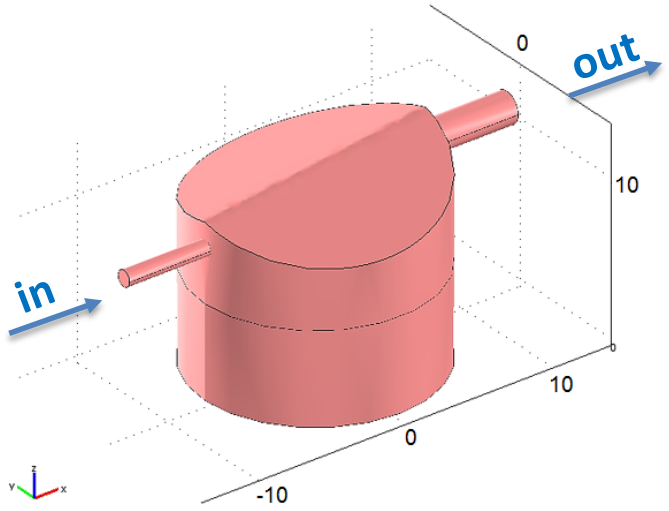
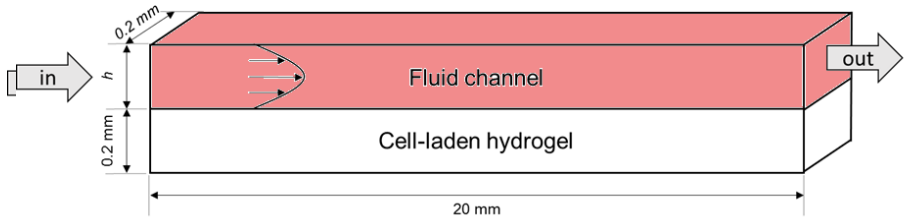


Milli VS Micro scaled systems

Steady-state multi-physics model which couples **oxygen mass transport and consumption** to **fluid dynamics** (solved in COMSOL Multiphysics 3.5a)

Fluid domain

(**O_2 convective** transport, **no consumption**)

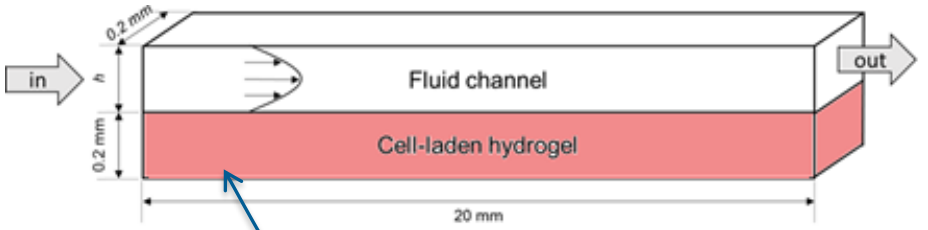


$$v_{in} = \frac{\tau_w \cdot h}{6\eta}, \qquad \tau_w = 15\ \mu\text{Pa}$$

- **h = 0.2 mm** --> v_{in} = **7.1×10^{-7}** m/s
- **h = 2 mm** --> v_{in} = **1.6×10^{-6}** m/s

Hydrogel construct domain

(**O_2 diffusion** and **consumption**)



$$R = \frac{V_{max} \cdot c}{K_m + c} \cdot \delta(c > c_{cr})$$

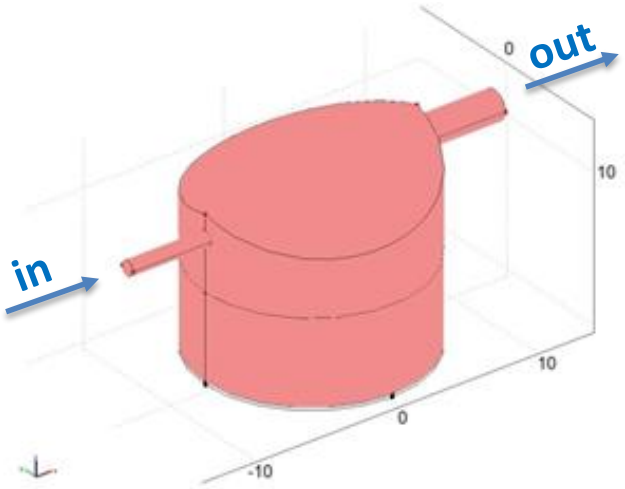
- **0.2 mm** thick gel
- **$7 \cdot 10^7$** hepatocytes/mL

Milli VS Micro scaled systems

Steady-state multi-physics model which couples **oxygen mass transport and consumption** to **fluid dynamics** (solved in COMSOL Multiphysics 3.5a)

Fluid domain

(**O_2 convective** transport, **no consumption**)

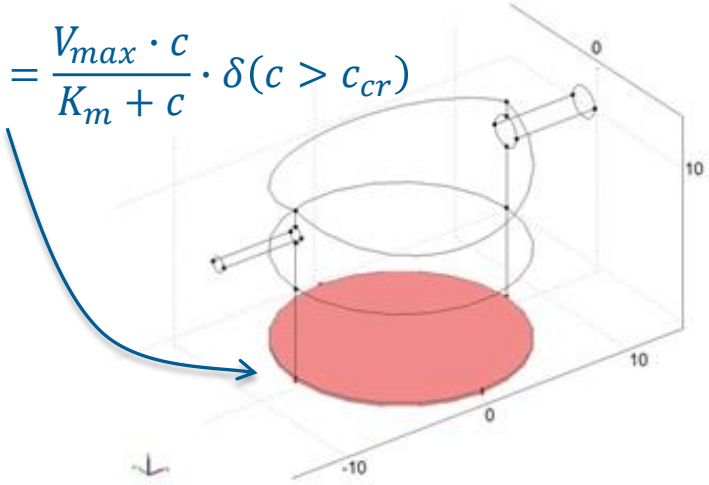


$v_{in} = 3.82 \times 10^{-3}$ m/s = **180 µL/min**
(from experimental results)

Hydrogel construct domain

(O_2 **diffusion** and **consumption**)

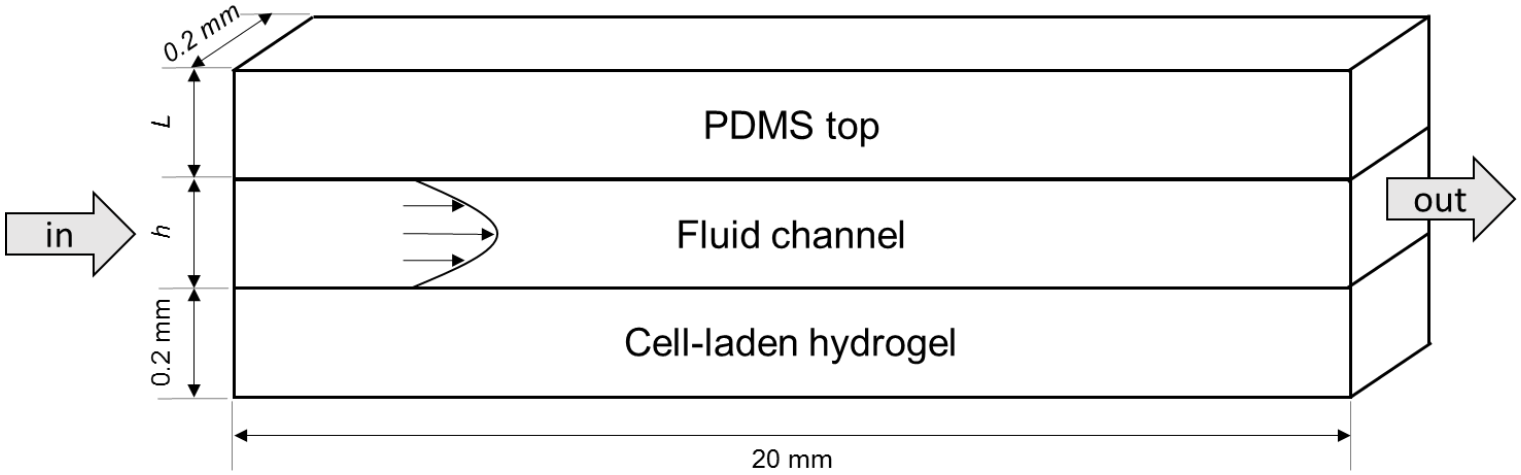
$$R = \frac{V_{max} \cdot c}{K_m + c} \cdot \delta(c > c_{cr})$$



- **0.2 mm** thick gel
- **$7 \cdot 10^7$** hepatocytes/mL

Milli VS Micro scaled systems

Most **micro-fluidic cell culture devices** are **made of PDMS**, a **gas permeable elastomer,** in order **to enhance oxygen mass transport**

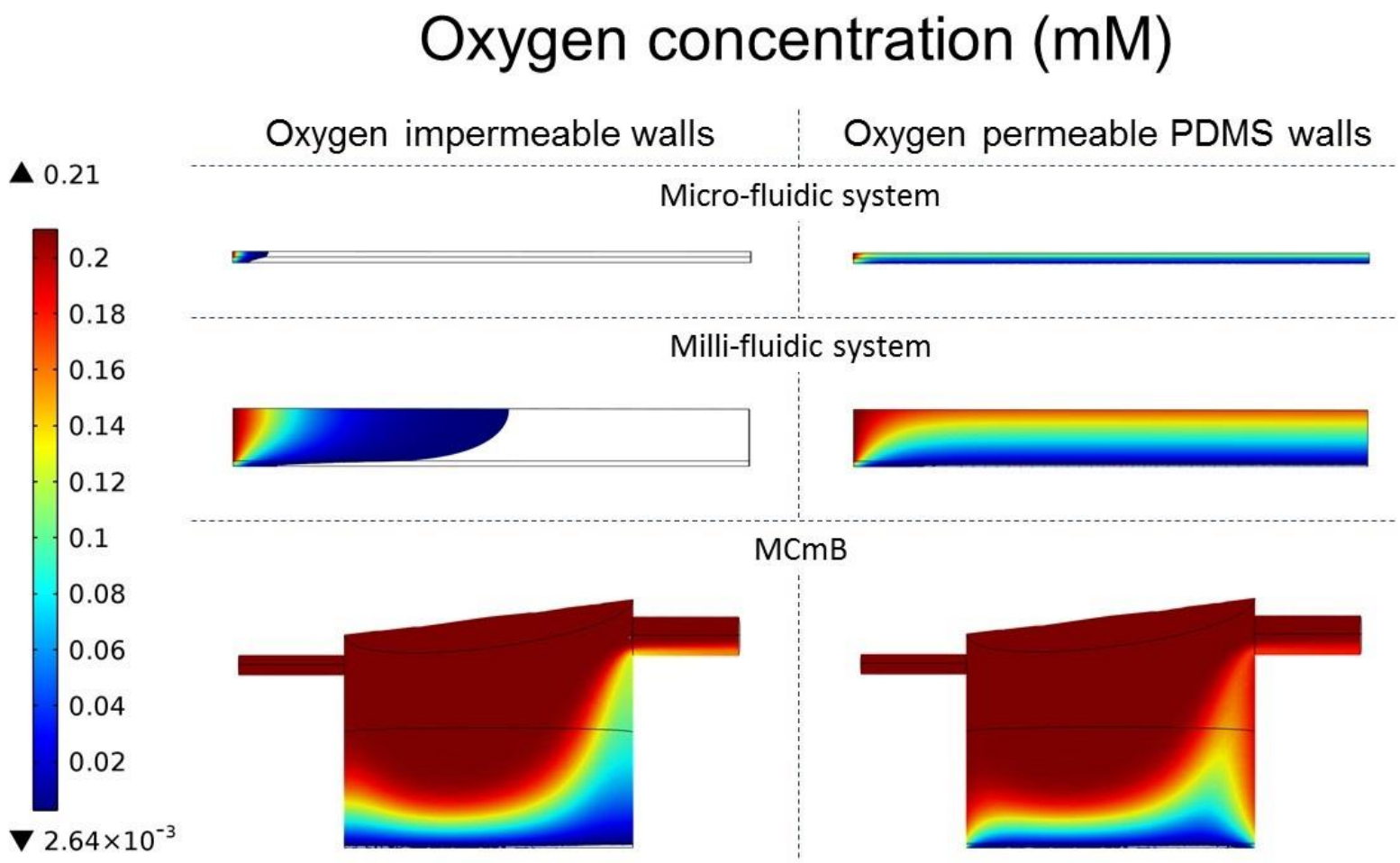


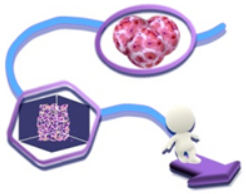
$$N_{O_2,PDMS} = K_{O_2}\left(p_{O_2} - \frac{c_{O_2}}{K_{H,O_2}}\right)$$

$$K_{O_2} = P_m/L$$

- **Micro-fluidic systems --> L = 5 mm** (typical)
- **MCmB --> L = 6 mm**

Milli VS Micro scaled systems





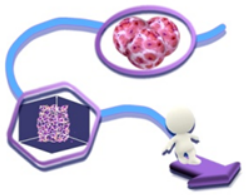
Milli VS Micro scaled systems

Modelled System	Micro-Fluidic		Milli-Fluidic		MCmB	
	OIW	OPW	OIW	OPW	OIW	OPW
Viable cells (%)	2.6	100	17.8	78	60.5	85.4

OIW: oxygen impermeable walls; **OPW:** oxygen permeable walls

O_2 diffusion through PDMS enhances the **viability of encapsulated hepatocytes,** especially **in micro-fluidic systems (PDMS over-reliance***)

* **Micro-fluidic cell culture devices** rely entirely on oxygen diffusion through the walls of the device, and **cannot be manufactured from oxygen-impermeable materials**, such as tissue culture plastic or glass



Milli VS Micro scaled systems

Configuration	Flow Rate (m^3/s)	R_{in} (mol/s)	Cell Number	R_{cons} (mol/s)	R_{in}/R_{cons}
Micro-fluidic	2.84×10^{-14}	1.42×10^{-13}	5.60×10^{4}	4.48×10^{-13}	0.3
Milli-fluidic	6.40×10^{-13}	3.20×10^{-12}	5.60×10^{4}	4.48×10^{-13}	7.1
MCmB	3.00×10^{-9}	1.50×10^{-8}	2.47×10^{6}	1.98×10^{-11}	758.3

R_{cons} = glucose cons. rate × cell number; **R_{in} =** glucose conc. (5 mM) × medium flow rate

Micro-fluidic systems may **suffer from depletion of nutrients** other than oxygen **if cell densities are increased to better approximate physiological values,** while **milli-fluidic systems** have **sufficient mass flow rate** to **ensure** an **acceptable R_{in}/R_{cons}** ratio

The MCB

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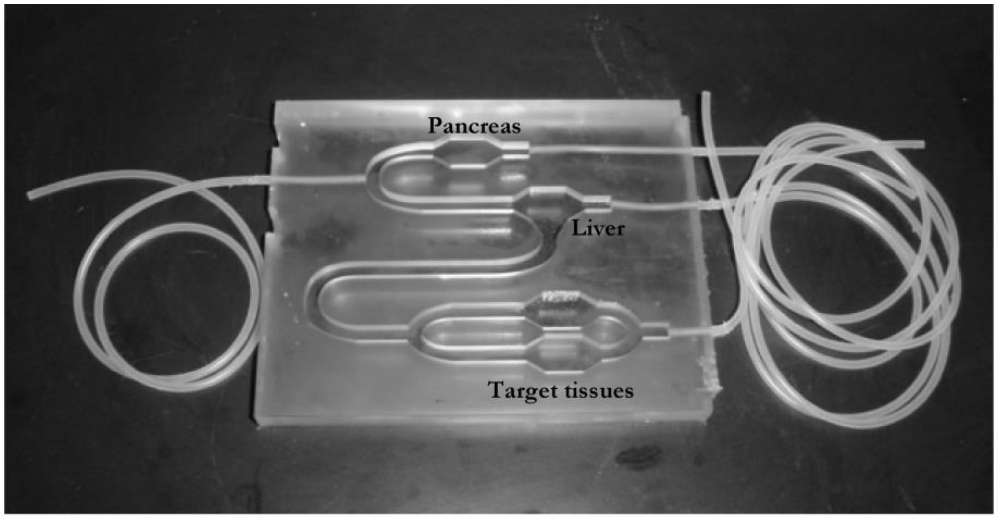
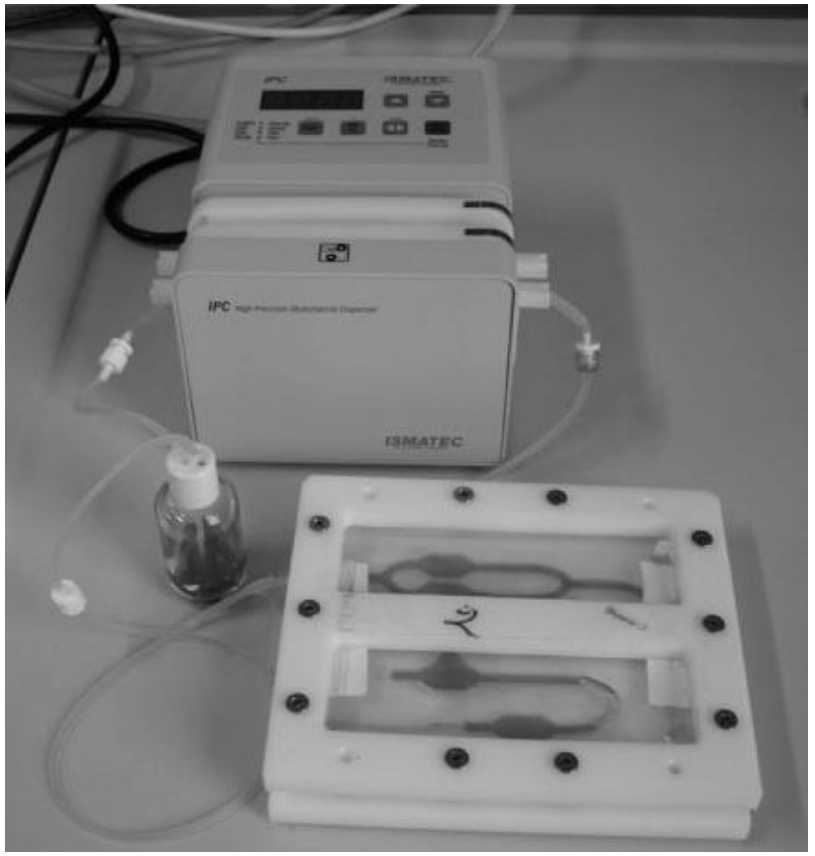

Pancreas
Liver
Target tissues


IPC
ISMATEC

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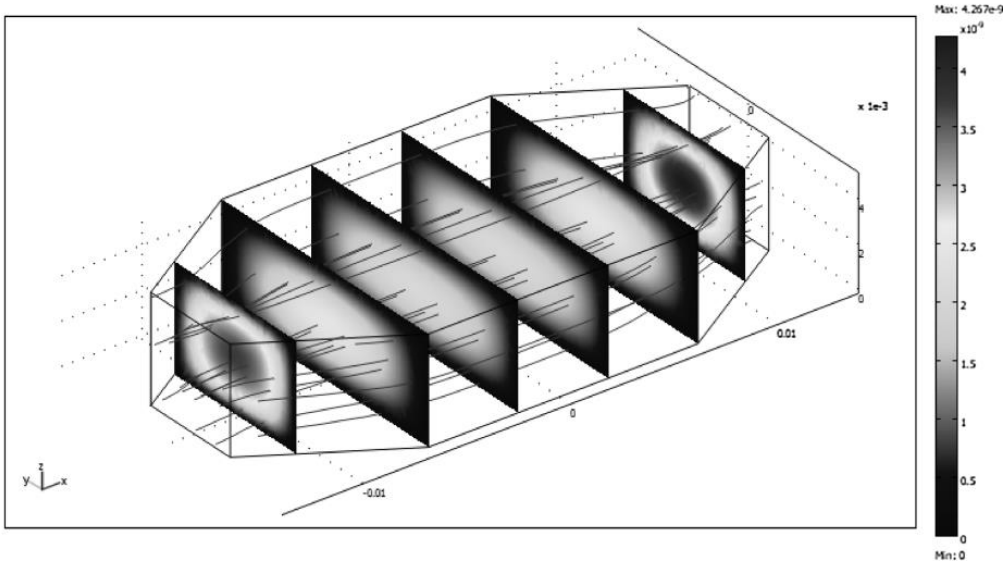
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4
3.5
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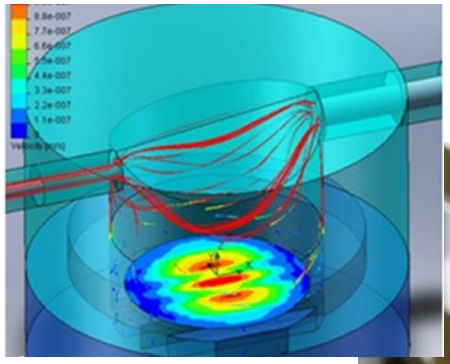
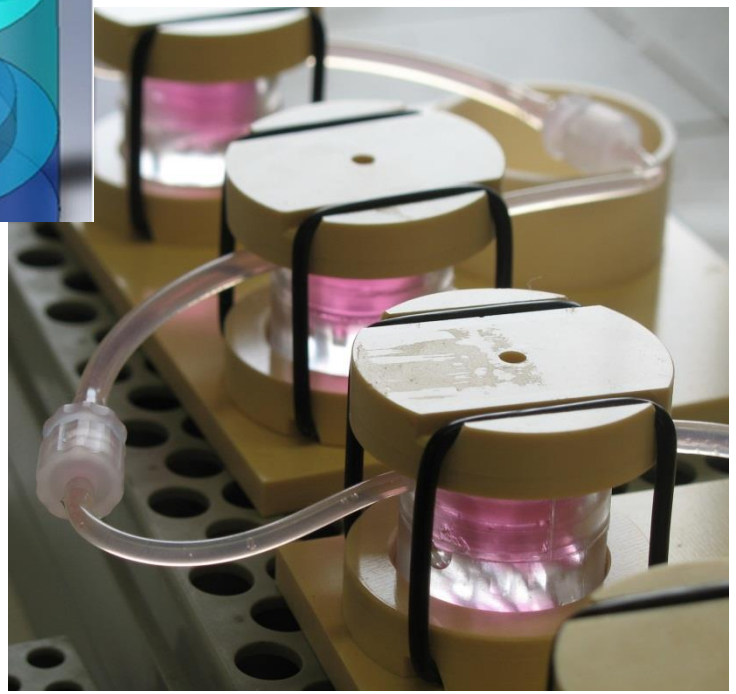
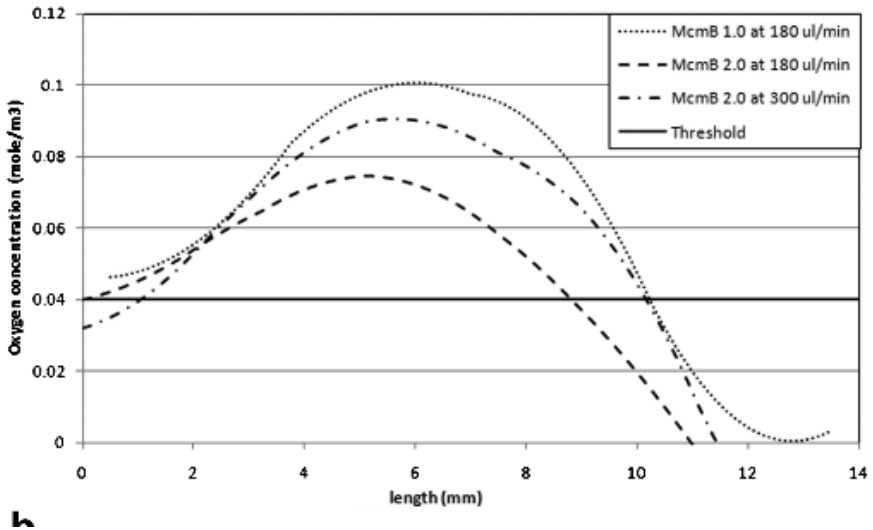
The MCmB

The QuasiVivo® (Kirkstall Ltd, UK) is a modular chamber which can be linked to other chambers for high throughput multi-compartment experiments

- High flow rates
- Low shear stress
- Connected cultures



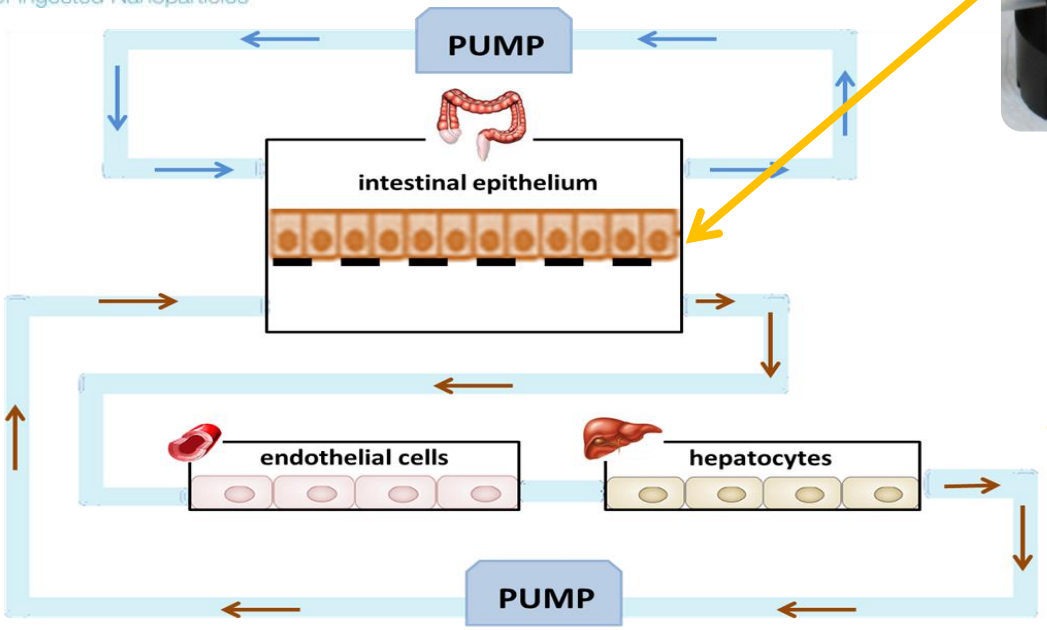


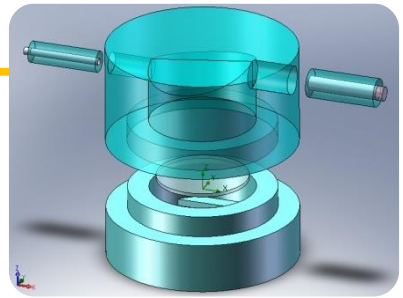


Connected Cell Culture

invitox
Toxicity of Ingested Nanoparticles

2 flow membrane bioreactor with realtime TEER



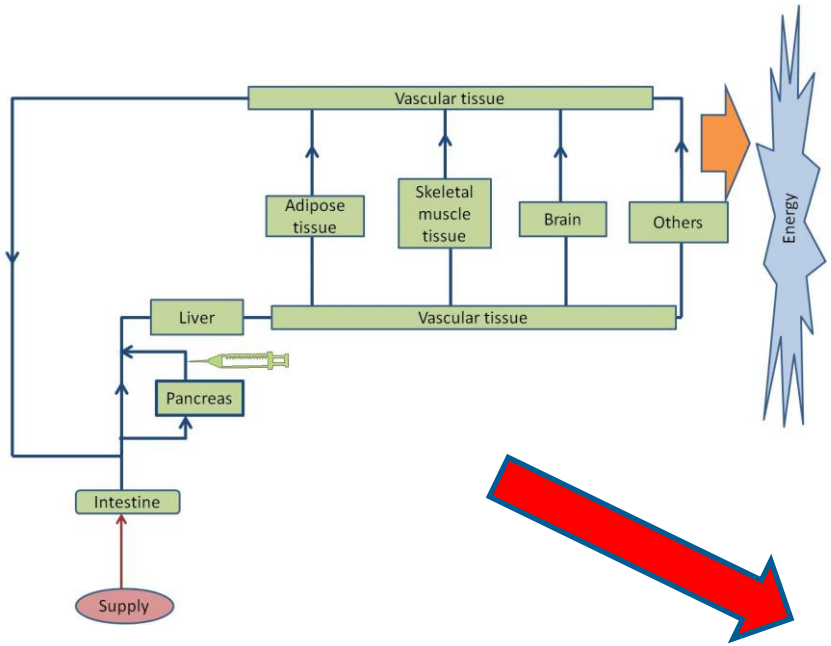


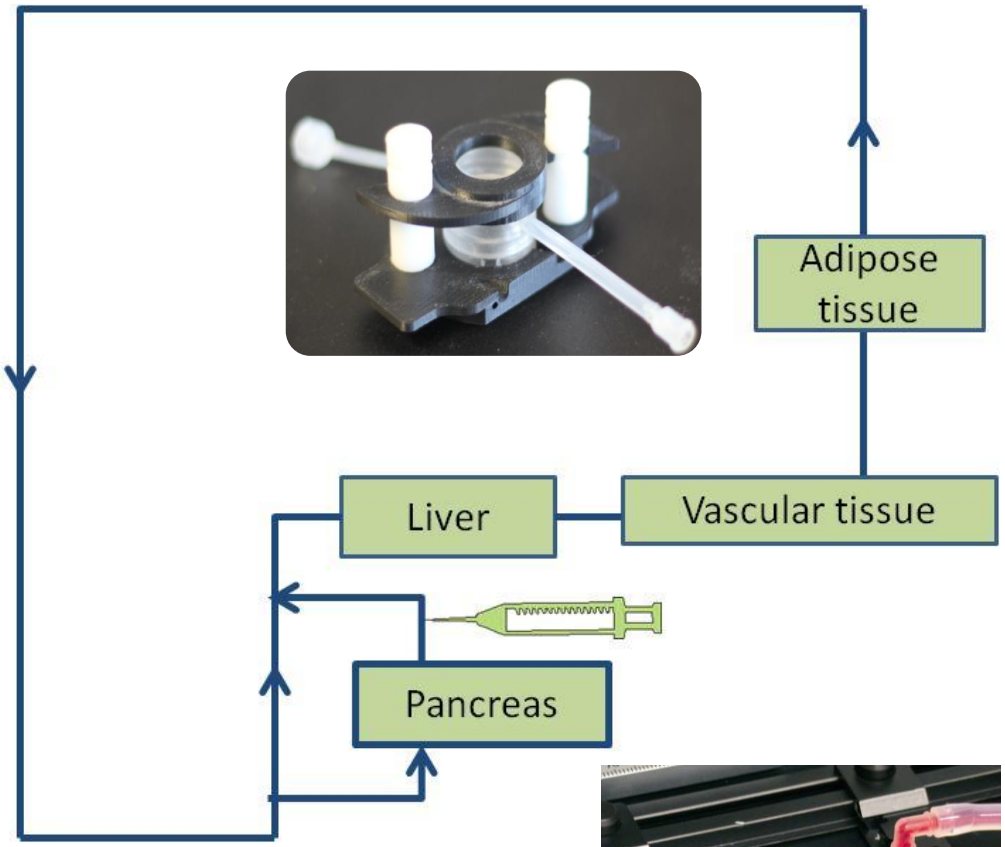
2 flow chambers

Assays:

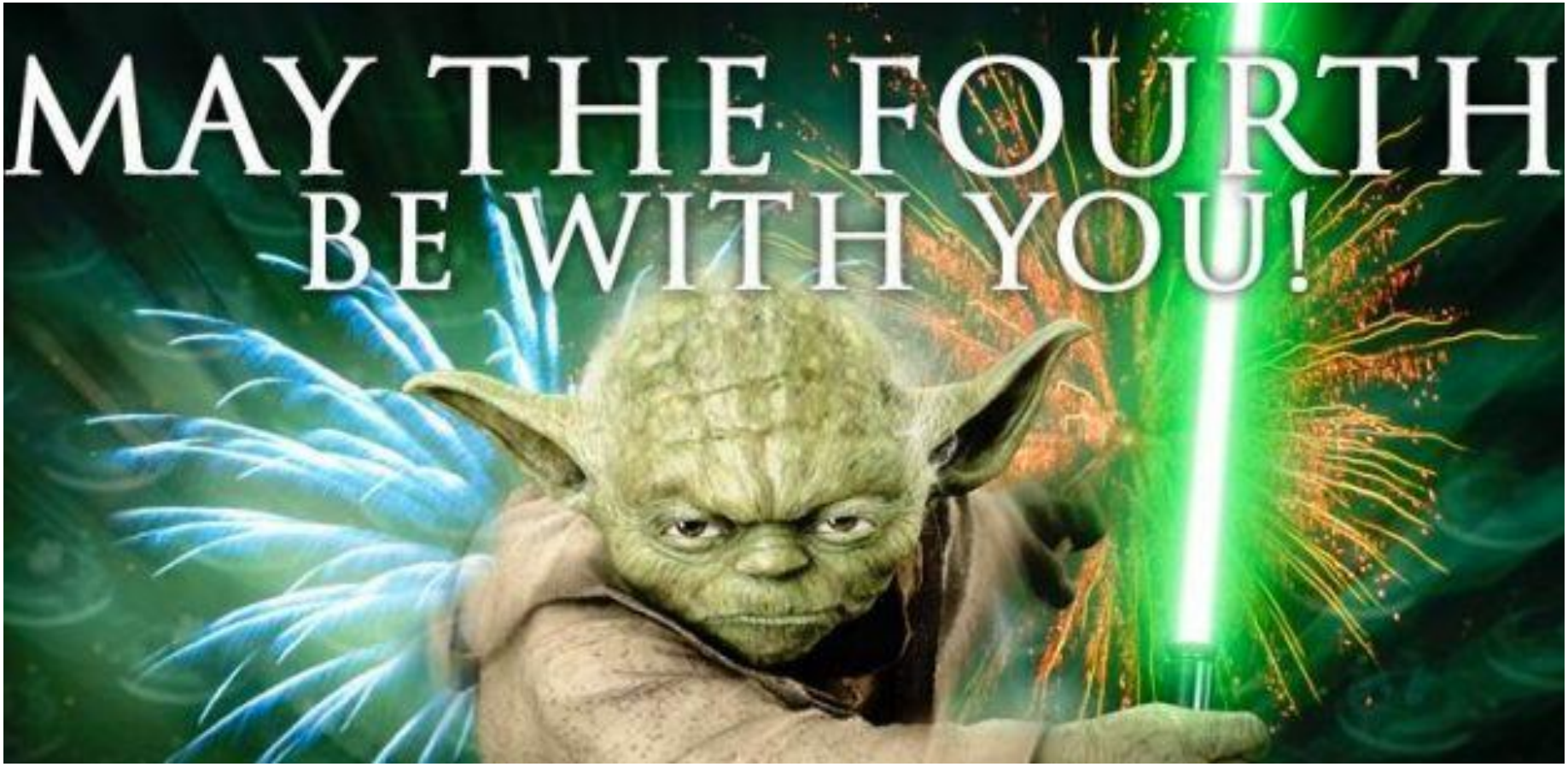
TEER, Alamar, LDH, vWF, cytokines, albumin, ZO-1, etc

Connected Cell Culture





Down-scaled and reduced to essential features of central metabolism


MAY THE FOURTH
BE WITH YOU!