Engineering a microphysiological system with controlled levels of oxygen as a platform for immunotherapies evaluation on liver metastatic tissues

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Context

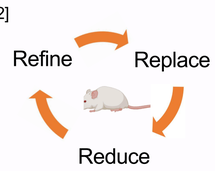
Colorectal cancer (CRC): in need of novel therapeutic approaches

- **2nd cause of cancer-related death, 3rd most diagnosed cancer** worldwide
- **50%** of patients will develop **liver metastasis**

Emergence of **Tissue Resident Memory** (T_{RM}) **T cell** therapy as a promising treatment for CRC liver metastasis [1]

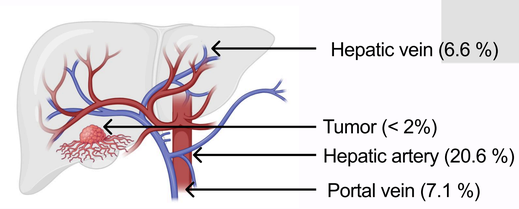
Microphysiological Systems (MPS), or **organ-on-chips**, as alternatives to conventionnal *in vivo* experimentation and 2D *in vitro* cell culture [2]

- ✓ Increased biological complexity
- ✓ Key biochemical and biophysical features
- ✓ 3D *in vivo*-like cells organization and interactions



Importance of oxygen control in cell culture

Most *in vitro* cell cultures are maintained under **normoxic** conditions (20 % O_2), whereas physiological tissues experience lower **physioxic** conditions (typically 3 - 7.4% O_2). In tumour, oxygen can drop to **hypoxic** levels (< 2% O_2) [3]. The **lack of oxygen control** may **bias experimental outcomes** [4].

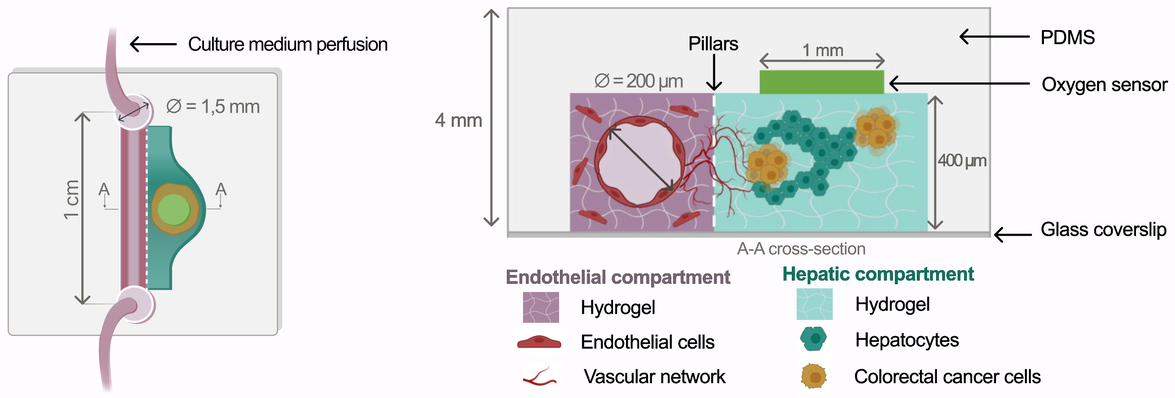


Heterogenity of oxygen levels in a human liver [5]

<u>Research problem</u>: How to design a MPS with controlled levels of oxygen to provide a physiologically relevant platform to study T_{RM} cell therapy effects on reconstructed CRC liver metastasis?

Design of the chip

Microfluidic chip reproducing a **perfusable metastatic-liver-on-chip** with a co-culture of endothelial cells, hepatocytes and cancer cells in hydrogels, separated by pillars, with **integrated oxygen sensing**.

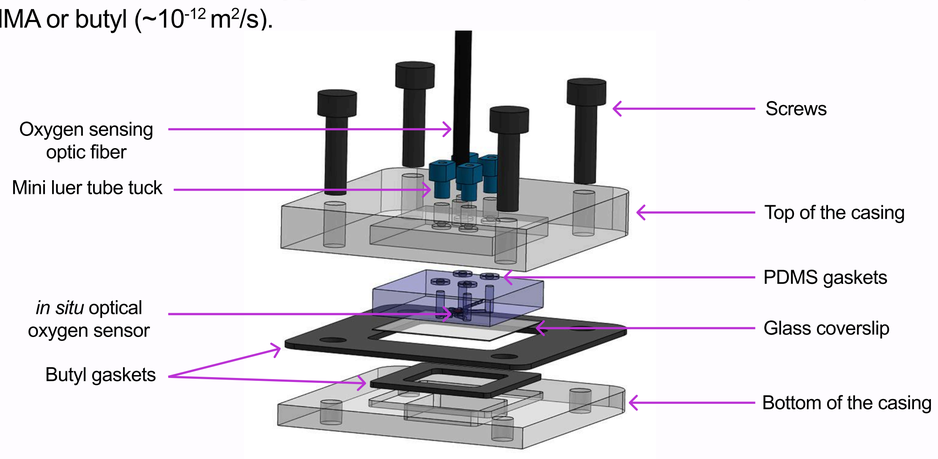


Schematic representation of the cell culture chip : Top view (left) and cross-sectional view (right) - *not to scale*.

Microfluidic chip made of PDMS (Polydimethylsiloxane) : **highly permeable to oxygen**
→ Need to find a way to **limit oxygen supply** to the chip

External control of oxygen levels

Design of a **casing** to limit the oxygen supply to the microfluidic chip, with the use of materials with a **smaller oxygen diffusion coefficient** than PDMS (~10^{-9} m^2/s), such as PMMA or butyl (~10^{-12} m^2/s).

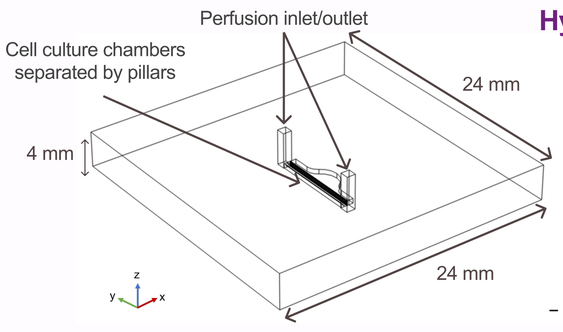


Split view of the design of a casing limiting the oxygen supply to the chip

Internal control of oxygen levels

1. Finite element simulations model

Numerical simulations using COMSOL Multiphysics



3D model on the microfluidic chip

Hypotheses:

- No oxygen supply to the chip other than perfusion of culture medium through endothelial lumen
- Consumption of oyxgen by the cells

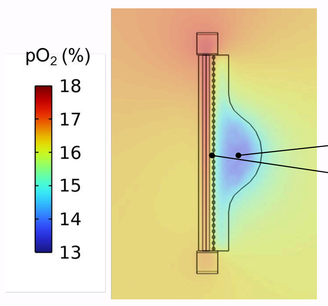
Michaelis-Menten kinetics

$$R_{O_2} = \frac{OCR \cdot N_{cell} \cdot C_{O_2}}{V \cdot (K_m + C_{O_2})}$$

- R_{O_2} : oxygen consumption of the tissue
- OCR : oxygen consumption rate of cells
- N_{cell} : number of cells
- V : volume of hydrogel
- C_{O_2}: concentration of oxygen
- K_m: Michaelis-Menten constant

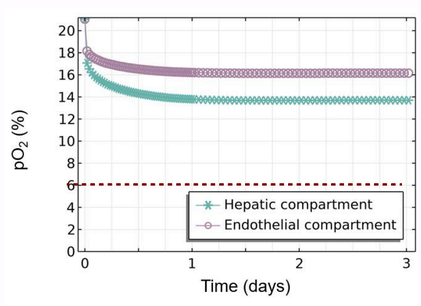
2. Time dependent study of the oxygen concentration

Study of the diffusion of oxygen through the device in the center of the tissue
→ Establishment of a stable **gradient of oxygen** due to **cellular respiration**



Top view of the device (z = 200 µm)
Oxygen gradient after 72 h

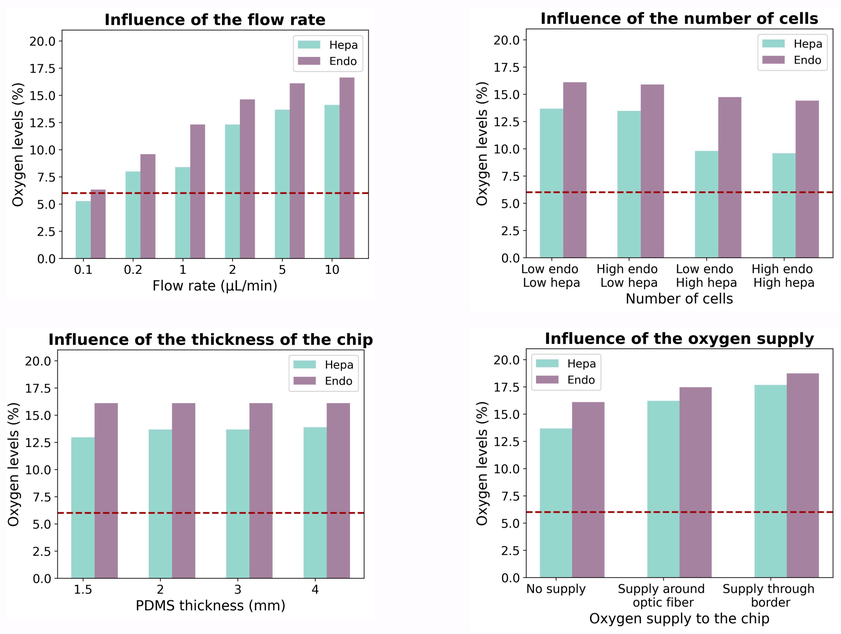
Study of 2 specific locations : in the **hepatic** and **endothelial** compartment



Target around 6% O_2

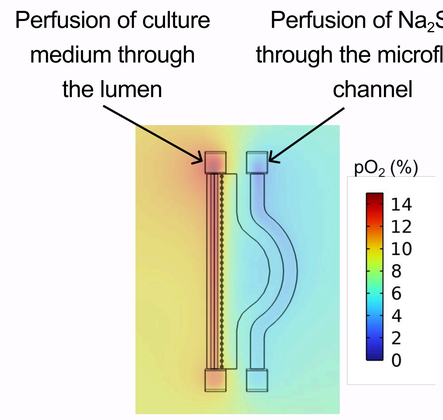
Evolution of the oxygen levels on chip with time

3. Control of steady-state oxygen levels



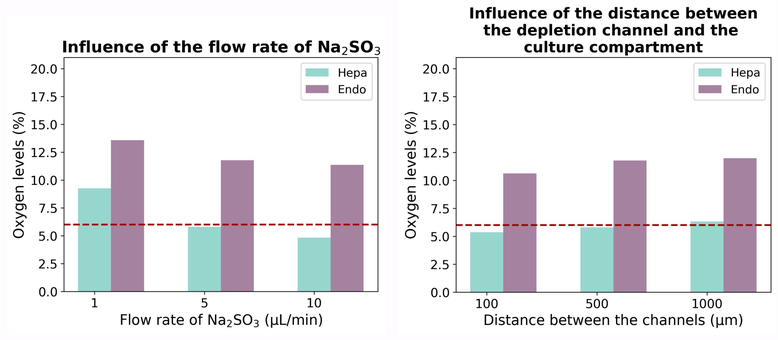
4. Addition of a depletion channel

Difficult to reach physioxic culture conditions: use of another microfluidic channel, flowing **oxygen scavenger** (Na_2SO_3) to further **decrease the oxygen levels** on chip.



Top view of the device (z = 200 µm)
Oxygen gradient after 72h, with depletion channel

Allows oxygen levels to reach physioxic conditions (< 6% O_2)



Several parameters can be changed to control oxygen levels on chip but successful limitation of the oxygen supply is essential.

Conclusion

- A **highly oxygen-impermeable casing** was designed to limit ambient oxygen diffusion into the microfluidic chip.
- Numerical simulations showed that the **perfusion flow rate** and on-chip **cells concentrations** are the most influential factors to decrease oxygen levels at the equilibrium.
- However, an **oxygen depletion channel** is required to reach **physioxic levels** in the metastatic compartment.

Perspectives

- Validation of the model with first experiments of oxygen sensing in the designed architecture.
- 3D co-culture of the different types of cells to measure their oxygen consumption.
- Monitoring of oxygen levels while cancer cells are exposed to T_{RM} cell therapy.

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