Engineering a microphysiological system of metastatic tissues as a platform for immunotherapies evaluation

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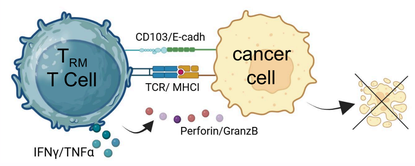
Context

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

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

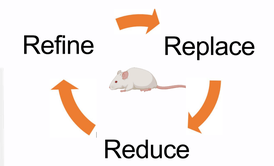
The current **preclinical models used during drug developement have shown limitations**, such as cost, interspecies variability, low predictive accuracy and ethical concerns [1]

→ Emergence of **Tissue Resident Memory** (T_{RM}) **T cell** therapy as a promising treatment for CRC liver metastasis [2]



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

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

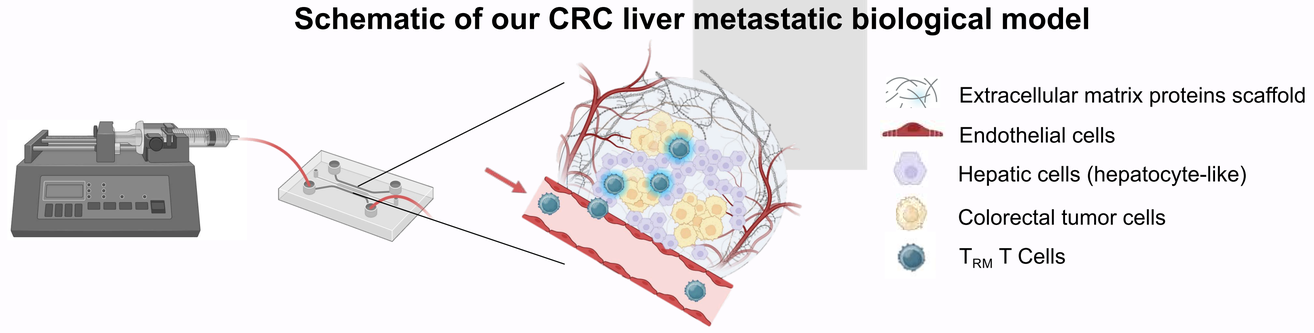


Research problem: can MPS provide a relevant platform to study cell therapy effects on reconstructed CRC liver metastasis?

Objectives

- **Model CRC liver metastasis in oxygen controlled microenvironment**
- **Reproduce multi-scale vascularization**
- **Study cancer cells response to a new T_{RM} cell therapy**

Schematic of our CRC liver metastatic biological model



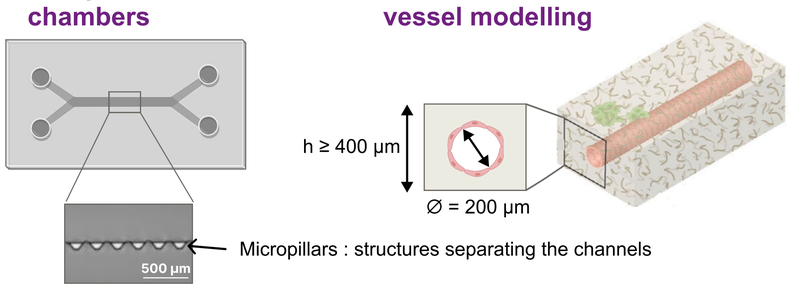
Results

1. MICROFABRICATION

Microchip features

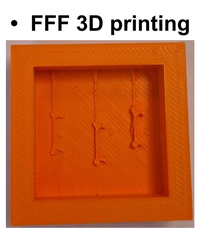
1. Compartmentalized chambers

2. Needle-based lumen for blood vessel modelling



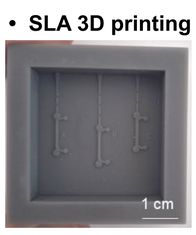
Mold microfabrication

- **FFF 3D printing**



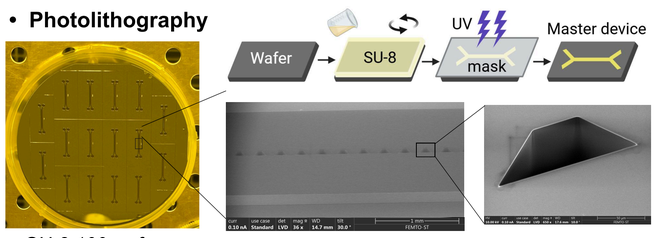
Fused Filament Fabrication

- **SLA 3D printing**



Stereolithography

- **Photolithography**



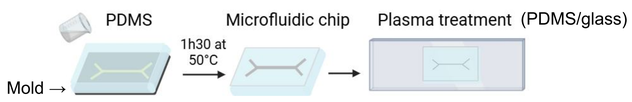
SU-8 100 wafer
h = 254 µm ± 10%

SU-8 micropatterns

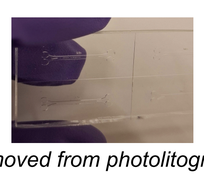
Holes in SU-8

Chip microfabrication

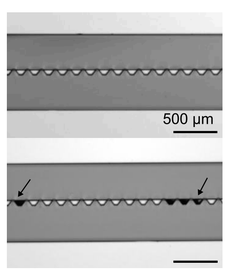
Soft lithography with PDMS



PDMS removed from SLA mold



PDMS removed from photolitography mold



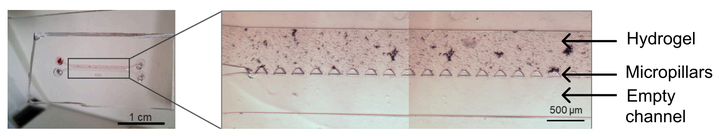
SU-8 wafer after PDMS removal

⚠ **PDMS adhesion** in **high aspect/ratio** micropatterns

2. HYDROGEL-ON-CHIP

1. Micropillars for compartmentalization

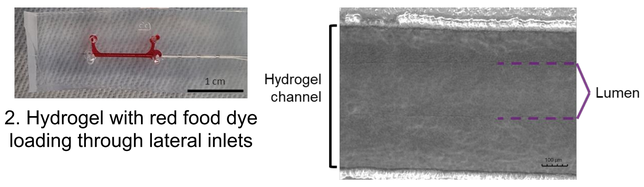
- PDMS soft lithography for 250 µm height channels chip fabrication.
- Hydrogel: **10 mg/mL fibrinogen, 1 U/mL thrombin**, in PBS.



→ Retention of **hydrogel in a single channel**
→ Correct order of magnitude for micropillars dimensions

2. Needle-based lumen in hydrogel

1. Needle (200 µm diameter) insertion in the microchannel



2. Hydrogel with red food dye loading through lateral inlets

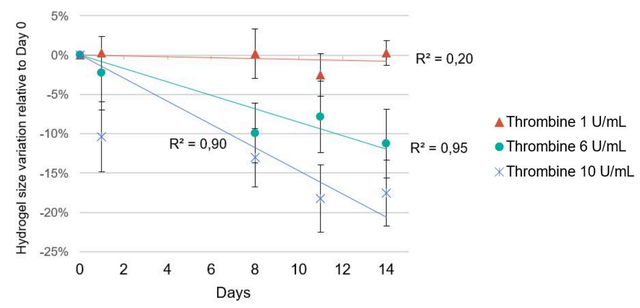
3. Needle removal

→ Successful **lumen formation**

3. MICRO-VASCULARIZATION

Hydrogel optimization

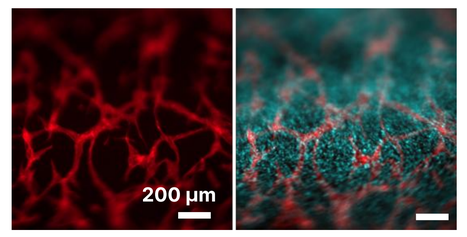
- Acellular 10 mg/mL fibrinogen gels drops (15 µL) kept in culture medium for 14 days
- Several **thrombin** concentrations tested : **1 to 10 U/mL**



→ **Thrombin 1 U/mL has best hydrogel stability over time**

Endothelial cells self organization

- **Co-culture HUVEC** ($1.65 \cdot 10^6$ cell/mL) **NHDF** ($3.3 \cdot 10^6$ cell/mL) for 7 days in 15 µL **hydrogel drops**, in EGM2:DMEM 1:1 culture medium
- Several **fibrinogen** concentrations were tested

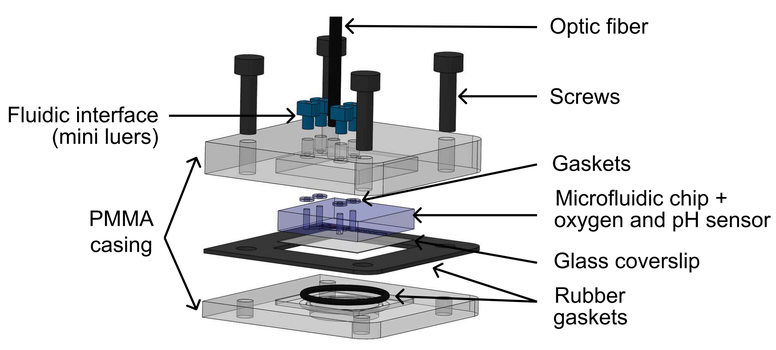


Immunofluorescence : CD31 (endothelial cells tight junctions) Hoechst (nuclei)

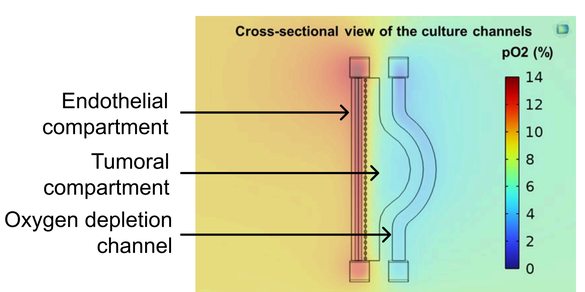
→ **Endothelial cells self organization in capillaries** has been obtained for 10 mg/mL fibrinogen, 1 U/mL thrombin gels.

4. OXYGEN LEVELS CONTROL

Casing to limit oxygen supply



Creation of an oxygen gradient



Numerical simulations (COMSOL Multiphysics)

Conclusion and perspectives

»» In terms of chip **microfabrication**, **photolithography** was the most relevant method to obtain high resolution microstructures. However, reaching 400 µm height for blood vessel modelling is still a challenge, and an additionnal step of **mold surface treatment** is required to enable full PDMS removal from the microstructures.

»» A **lumen in hydrogel-on-chip** was succesfully formed. The next step is to study its perfusability and seed liver endothelial cells to **mimic a perfused liver sinusoid.** Moreover, micro-vascularization has to be reproduced with **liver endothelial cells** and on-chip, in order to increase the CRC liver metastasis model biomimetism.

»» The **design of the microfluidic chip will enable oxygen levels control** by limiting gazeous exchange and by flowing an oxygen depletion solution near the cells.

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References

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[2] S. Abdeljaoued et al. OncoImmunology 14, 2455176 (2025).