



# DEVELOPMENT OF VASCULARIZED TUMOR-ON-CHIPS

Toward a physiological microsystem modeling  
the glioblastoma and blood-brain barrier.



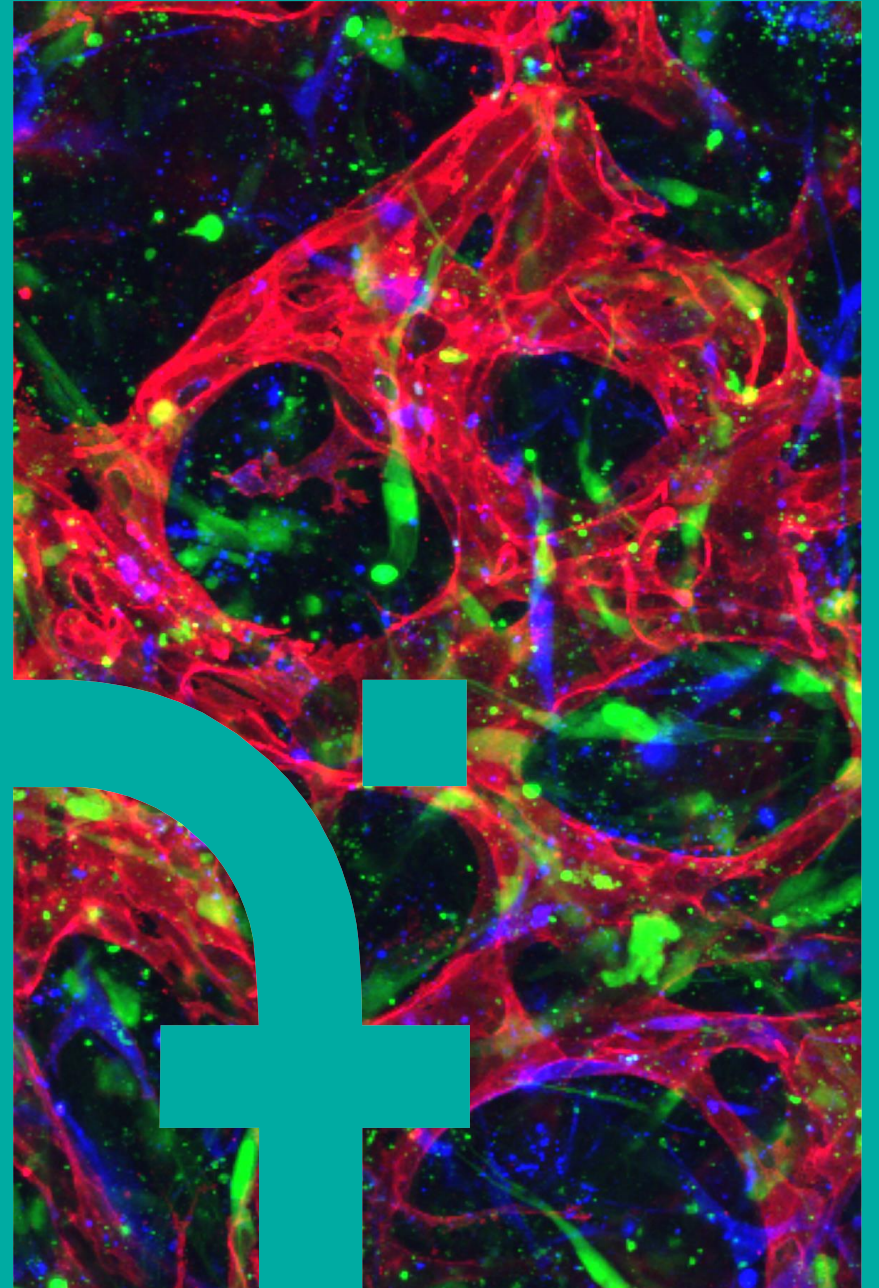
Agathe Figarol, Forum du Cancéropole Est, 10/11/2023

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Département MN2S : Micro Nano Sciences et Systèmes

Equipe BIND : BioInterfaces aNd Design

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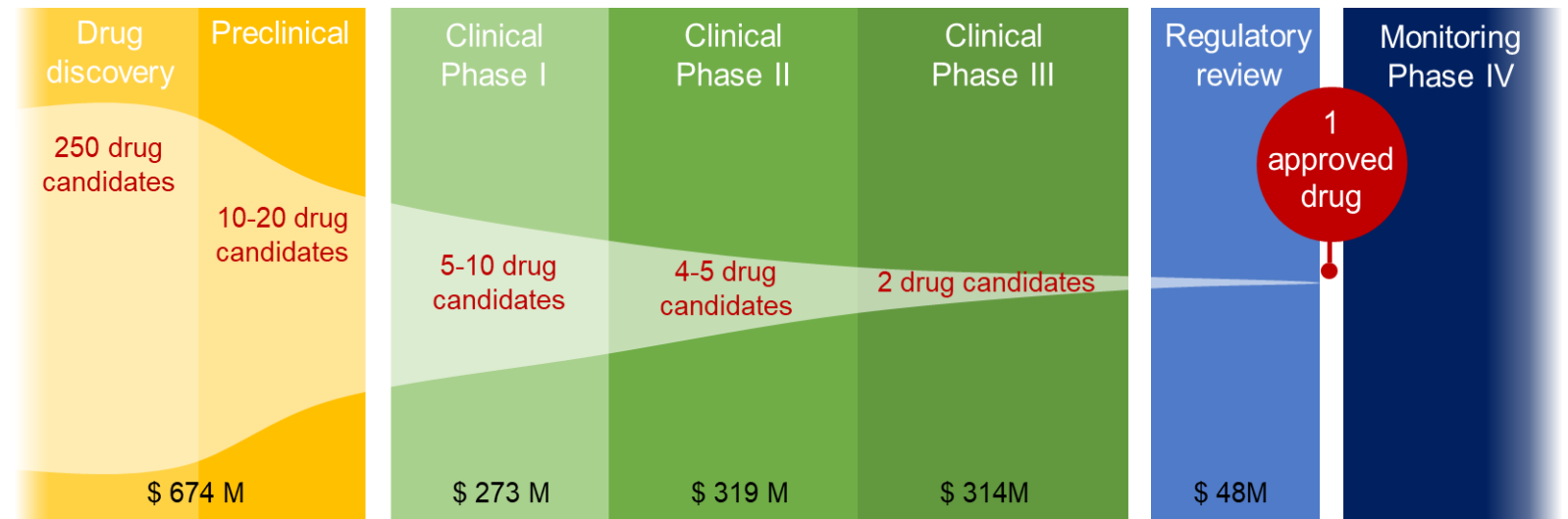
13<sup>e</sup> FORUM 2023  
CANCÉROPÔLE Est

# CONTEXT

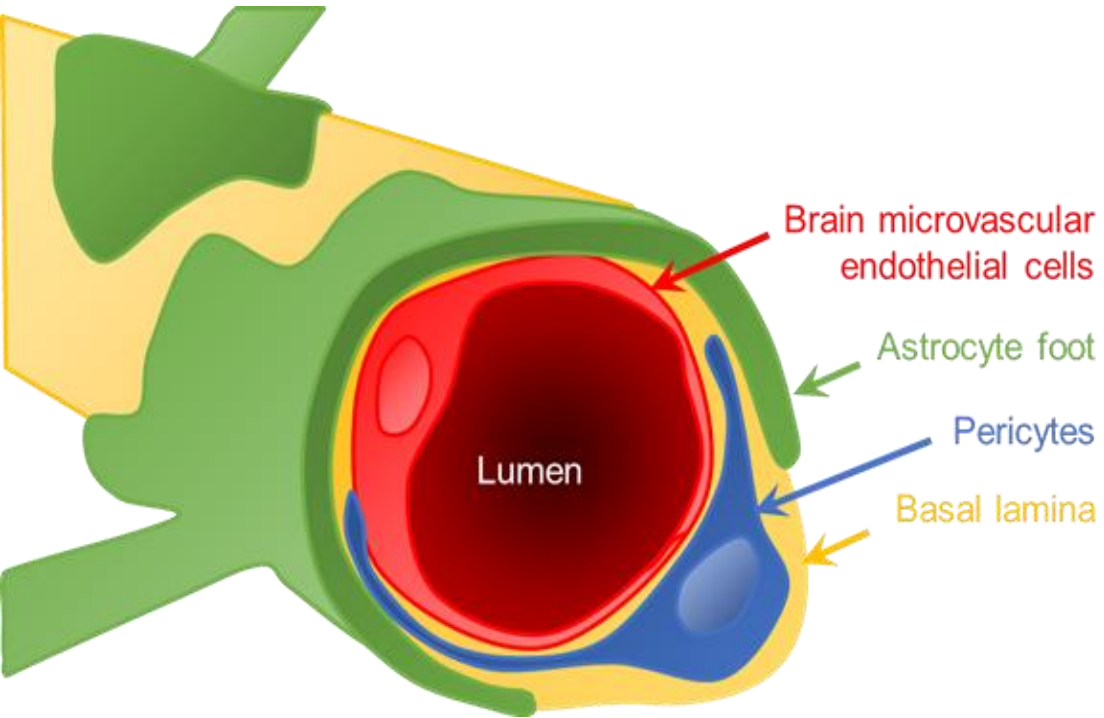
The valley of death of pharmaceutical drug development  
≈ 90% transition failure from bench to bed

Conventional preclinical models lack pertinence

- Poor clinical projections in patients
- Time and money consuming



# CONTEXT

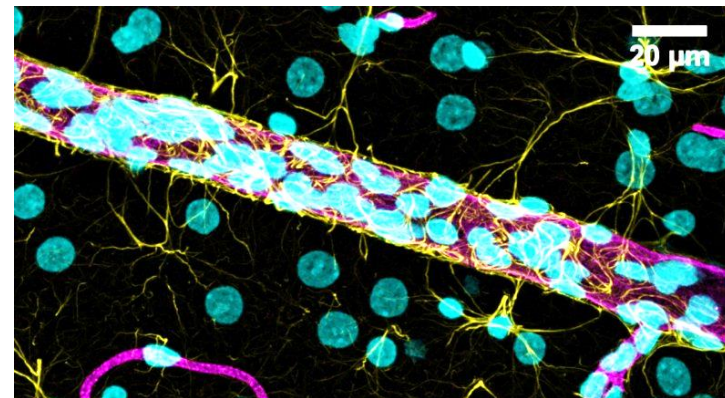


## Drug development

- For mental and neurologic diseases
- Have twice the risk of failure

The **BBB** : blood brain barrier blocks the transport of

- 100% of large pharmaceutical drugs
- 98% of small pharmaceutical drugs



# CONTEXT

The **GBM** : glioblastoma multiforme

- the most frequent, and
- the most aggressive brain cancer

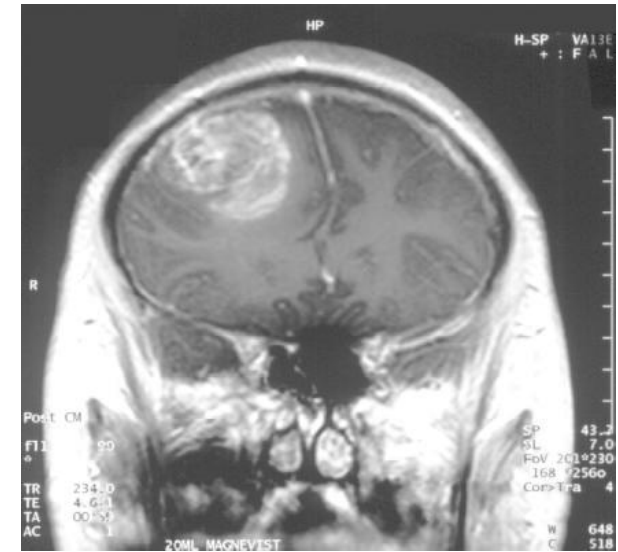
1% of all cancers → 2% of cancer-linked mortality

- surgery + radiotherapy + chemotherapy (TMZ)
- 12 to 18 months of life expectancy

Lot of expectations on nanocarriers

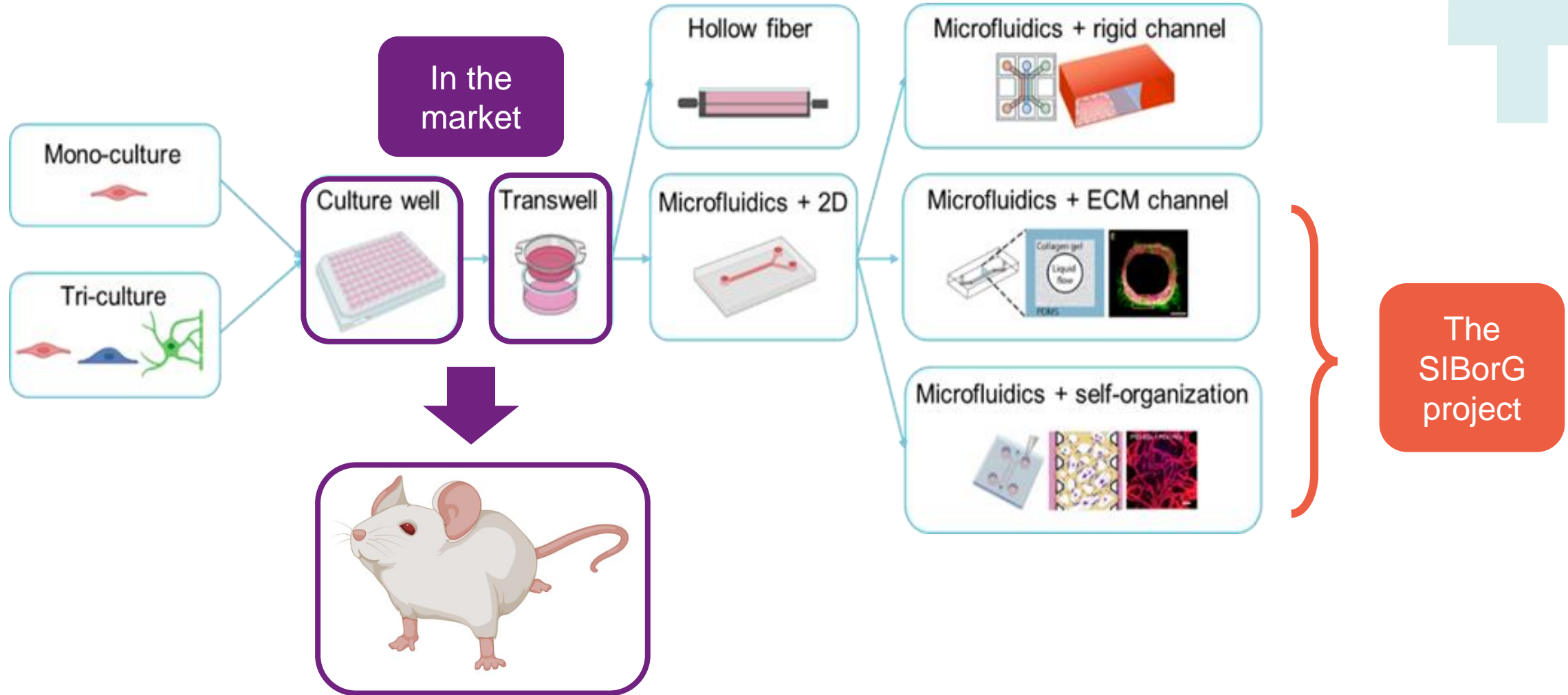
- Could facilitate the transport through the BBB
- Can reduce the drug toxicity

But < 1% nanodrugs reach the tumor & the transition to clinic is still a challenge.



*MRI scan of a GBM in a 15 year old*

# PRECLINICAL BBB AND GBM MODELS



# A GLOBAL PROBLEMATIC

## ↗ neurologic pathologies

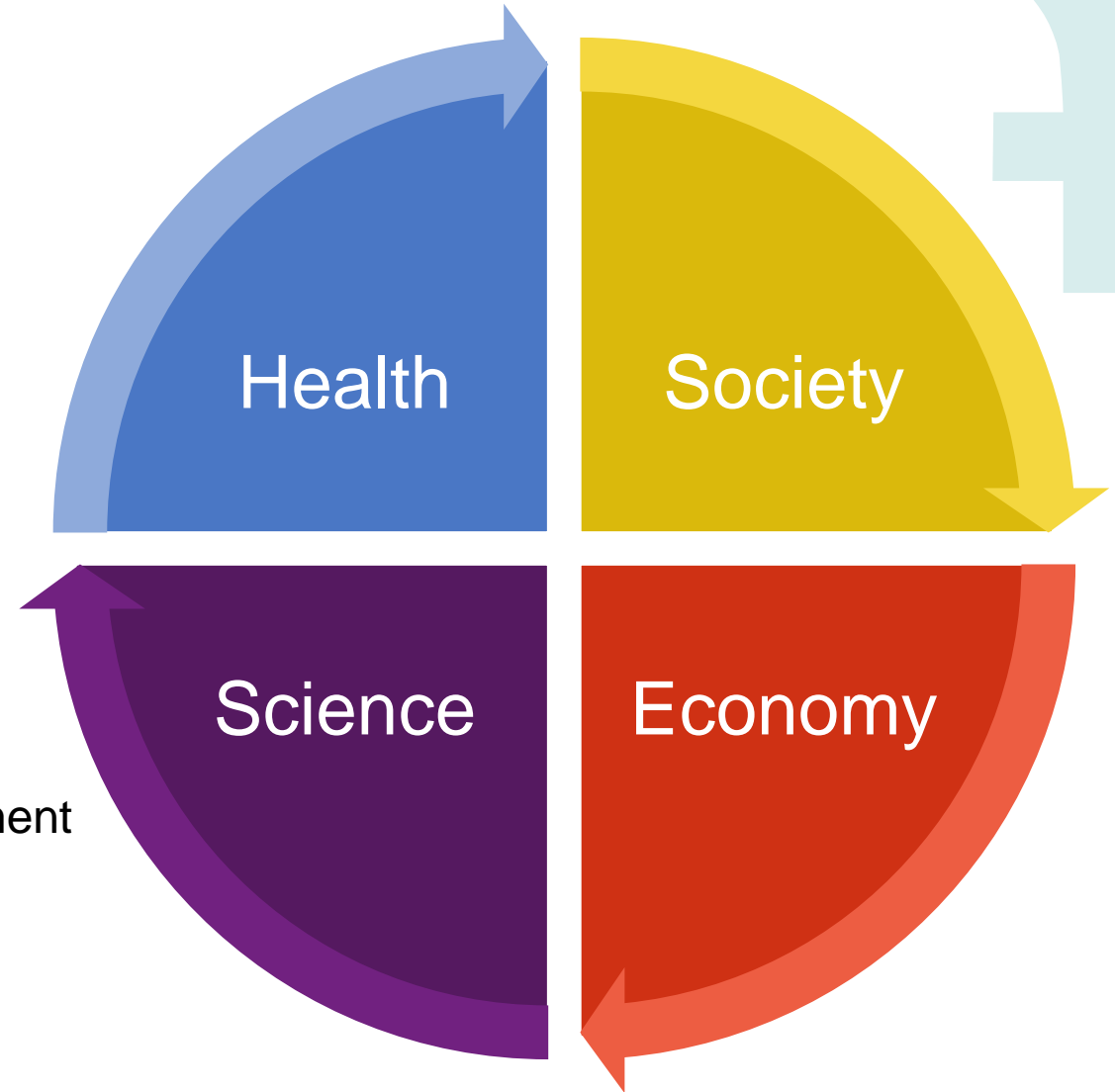
- + 17,3% of brain and central nervous system cancers (1990-2016)
- 7,7 millions DALYS (disability-adjusted life years)

## 10 millions animals / year in EU

- Directive 2010/63/EU
- 3Rs

## High industrial expectations

- Microfabrication allowed organ-on-chips development
- Pharmaceutical pharmacy are waiting for efficient alternatives



# THE SIBorG PROJECT & METHODOLOGY

Goals: ↗ clinical transfer rate  
↗ innovative therapies against brain cancer

Objective: a new preclinical model to screen drug candidates for transport and therapeutic efficiency

- I. Optimization of a **hydrogel for 3D BBB vascularization** modelling
- II. Development of a **vascularized GBM tumor-on-chip**

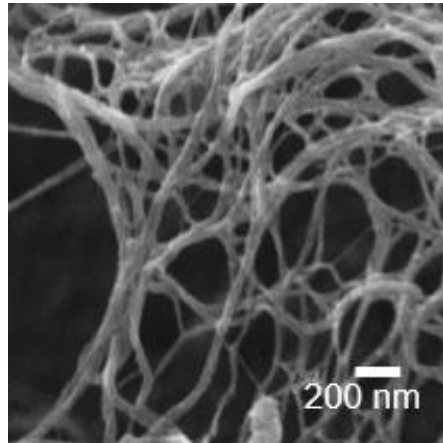
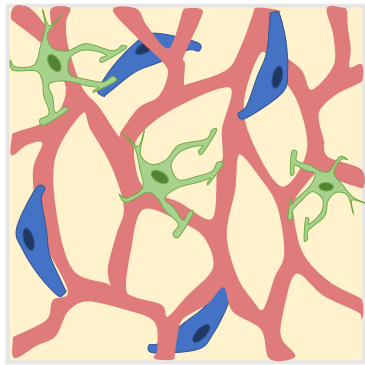
# I. OPTIMIZATION OF A HYDROGEL FOR 3D BBB VASCULARIZATION MODELLING





*Post-doctorate in Osaka University*



# 3D CULTURE IN A HYDROGEL

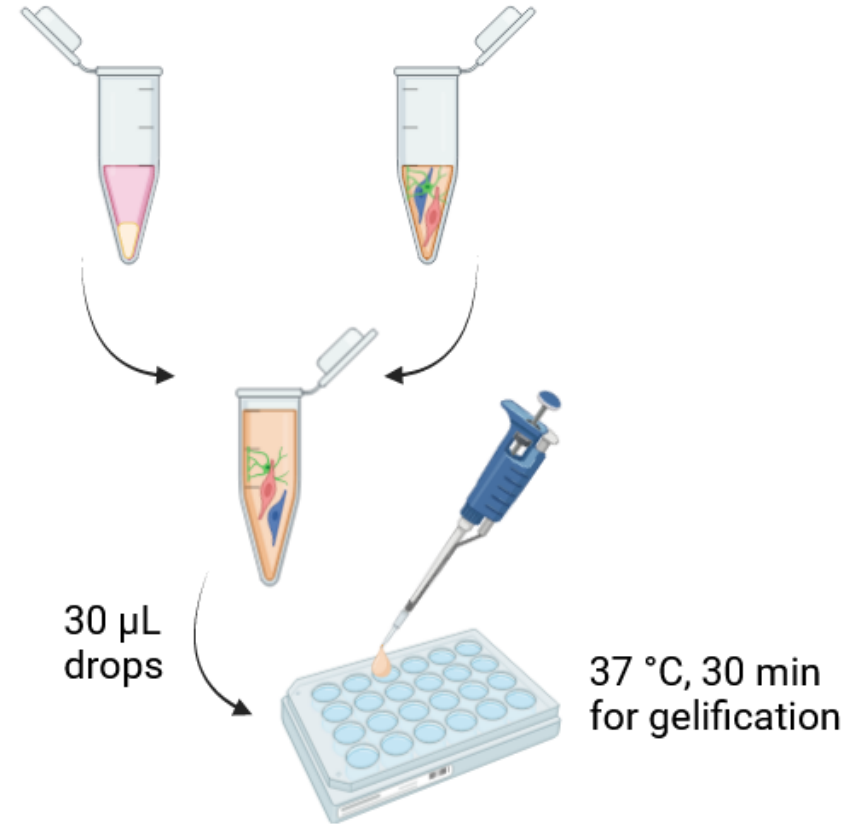
Osaka University, Chemical engineering department, Matsusaki's laboratory



-  Hydrogel : collagène de type I, fibrine
-  Cellules endothéliales
-  Péricytes
-  Astrocytes

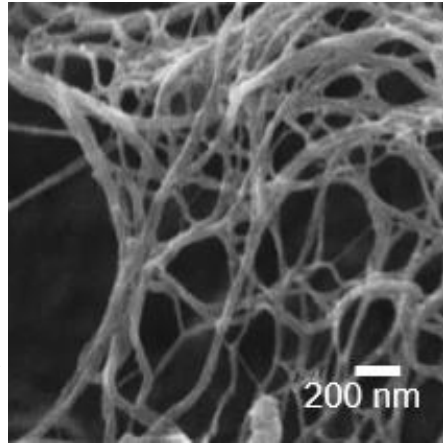
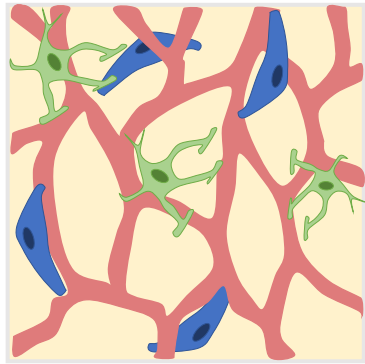
Collagen (17 mg/mL)  
+ fibrinogène (6,7 mg/mL)  
+ medium





Cells ( $5,8 \cdot 10^6$   $\mu$ /mL)  
+ thrombin (10 U/mL)  
+ medium



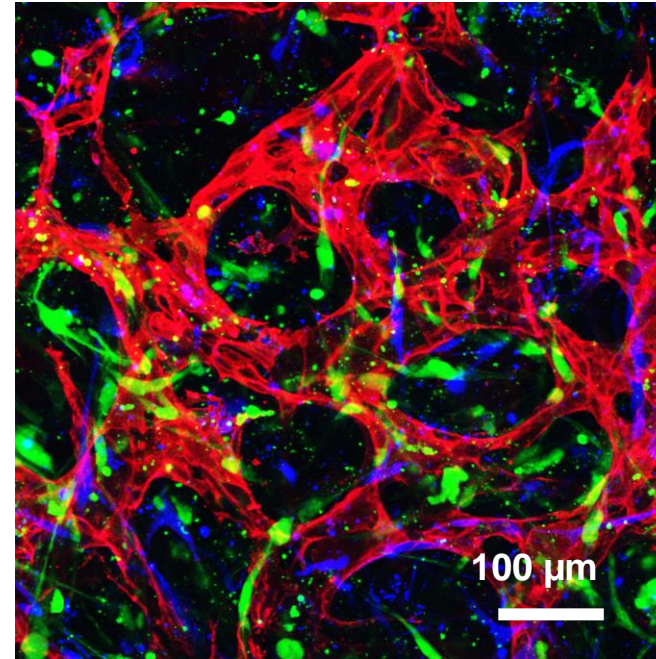
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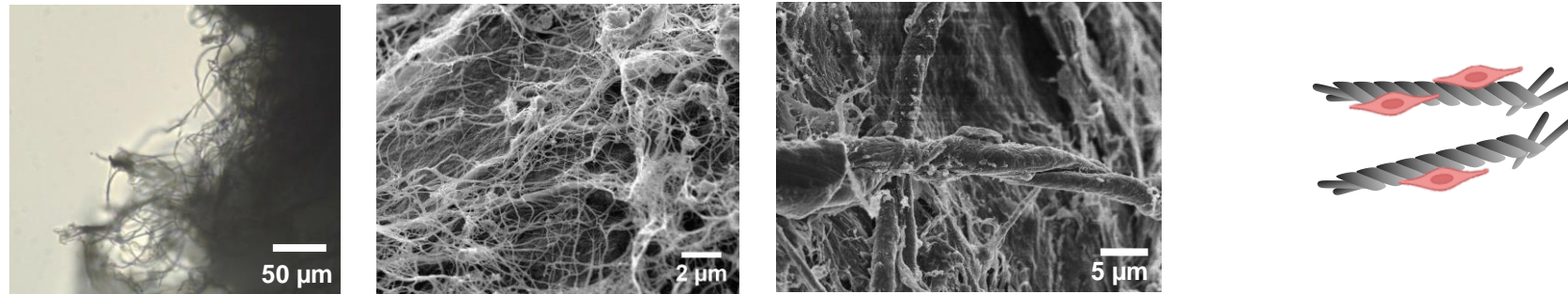
7 days later  
Incubation at 37°C, 5% CO<sub>2</sub> with medium change at d3



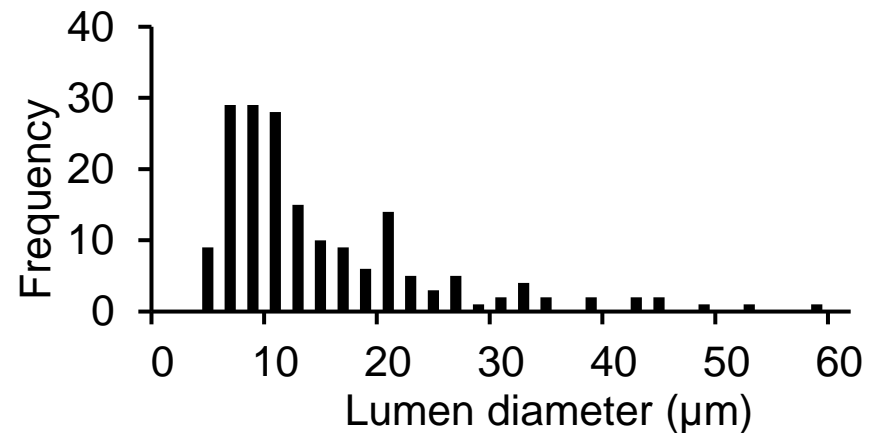
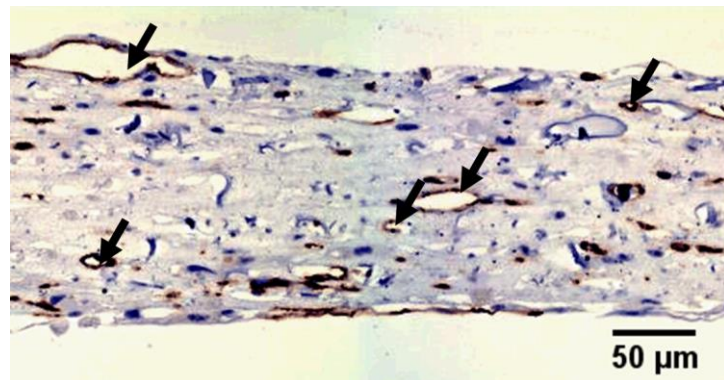
Self-organization in a 3D network of brain microvasculature

# Validation of the BBB vascularization

- Collagen and fibrin fibrous gel (SEM)

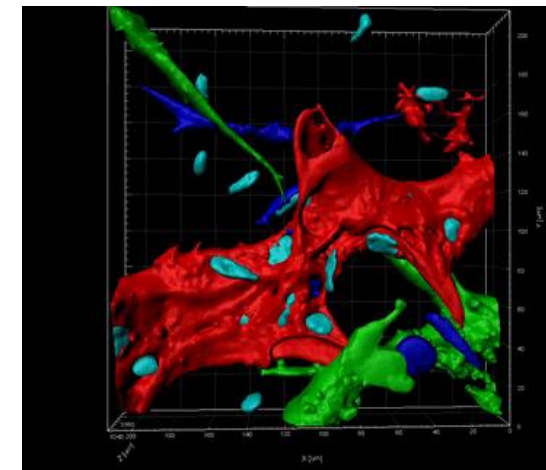
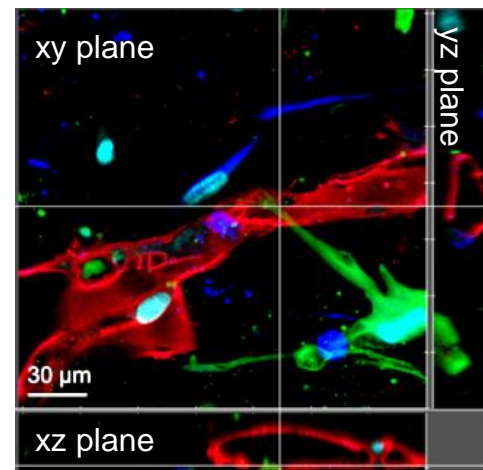
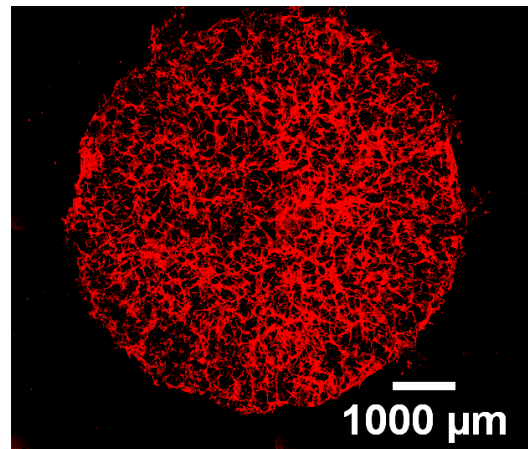
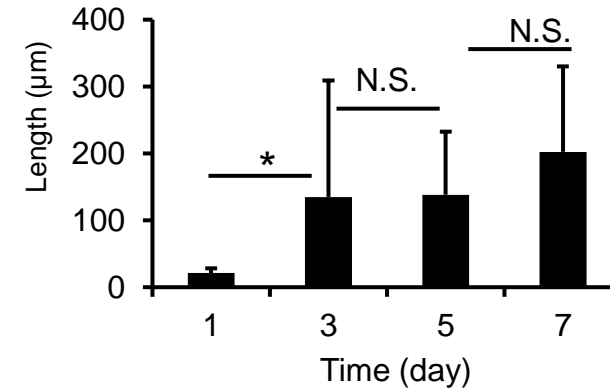
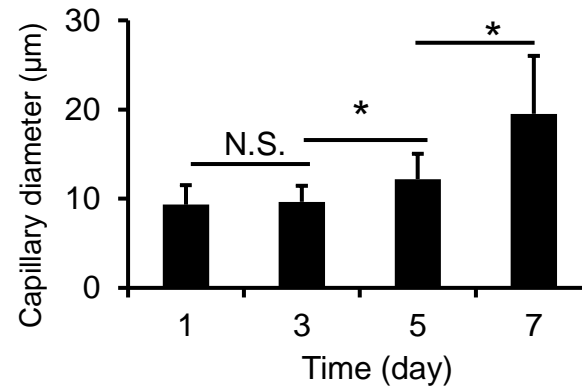
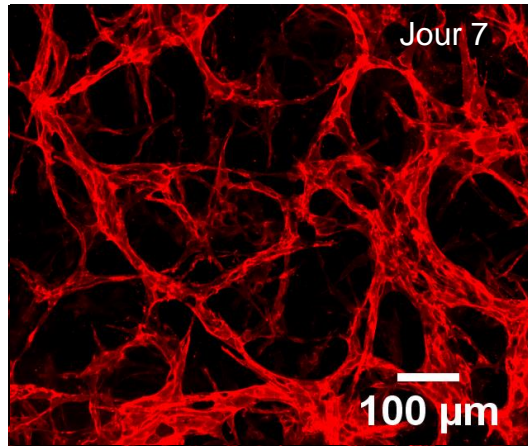


- Capillary lumen (immunohistochemistry CD31 for tight junctions of endothelial cells)



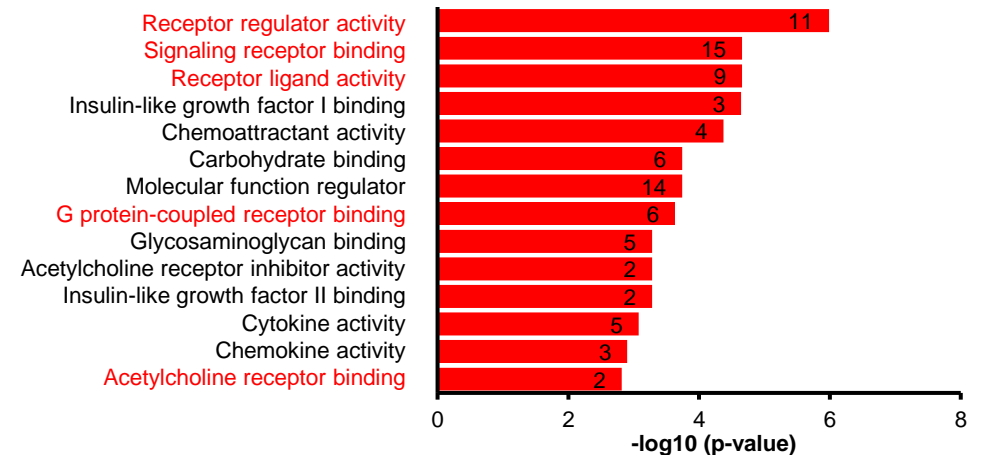
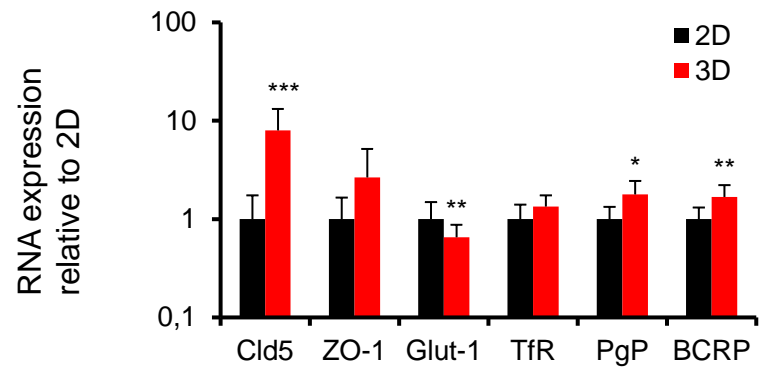
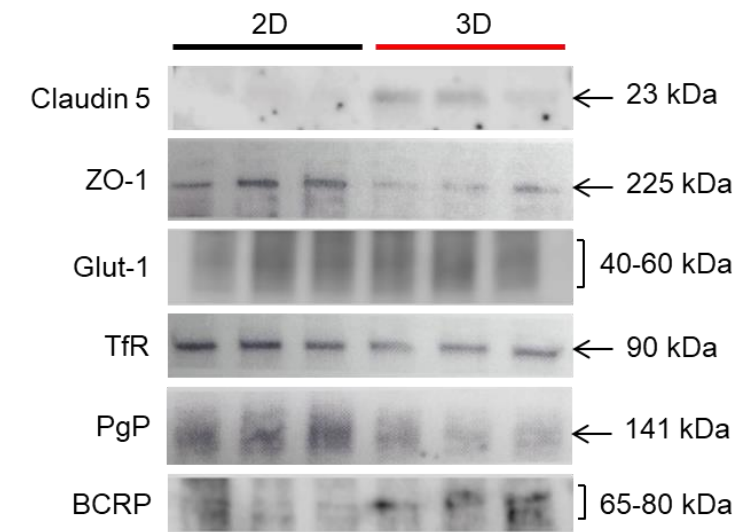
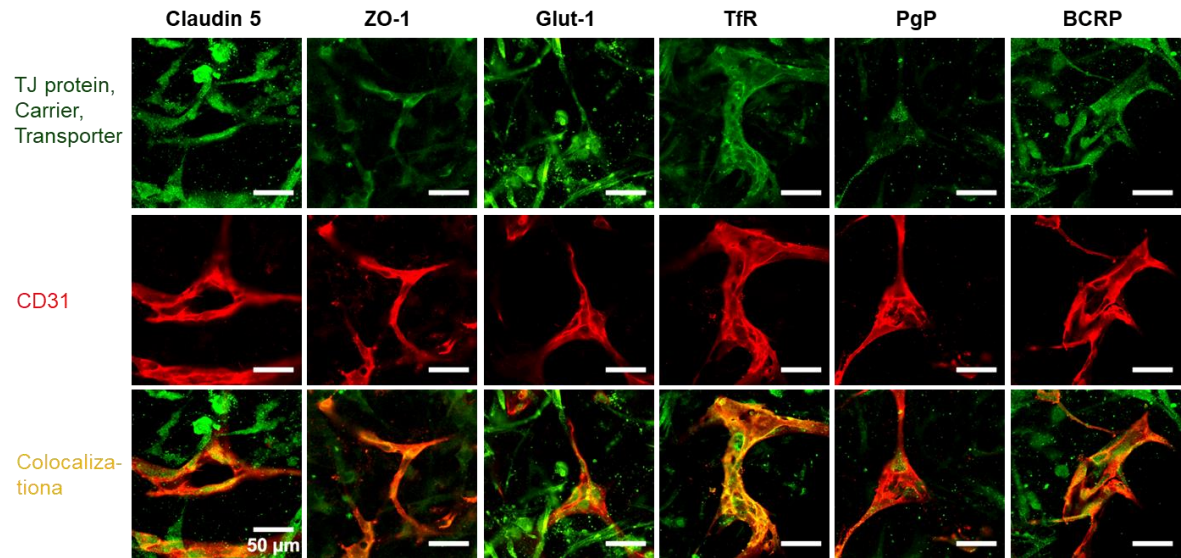
# Validation of the BBB vascularization

- Capillary network (immunofluorescence **CD31 for endothelial cells**, **cell tracker for pericytes**, **cell tracker for astrocytes**, **Dapi for nuclei**)

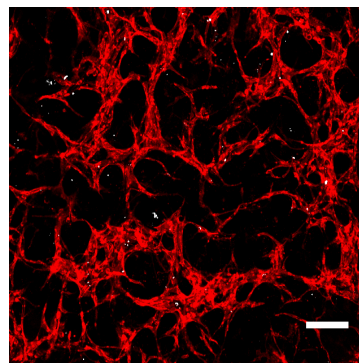
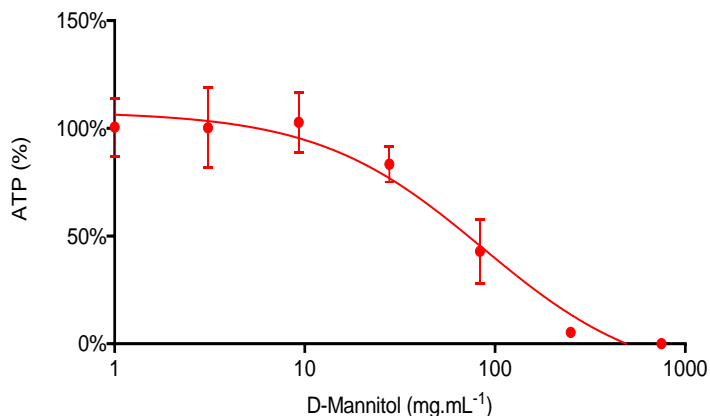


# Validation of the BBB vascularization

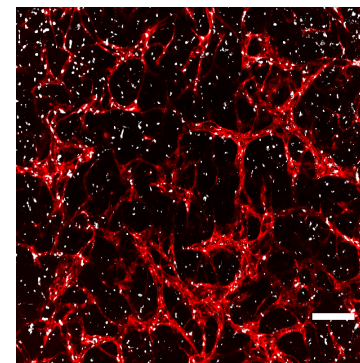
- Protein and gene expression (immunofluorescence, Western blot, PCR, transcriptomic)



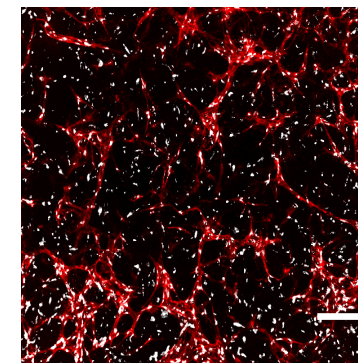
# Direct possible applications in toxicology



Negative control

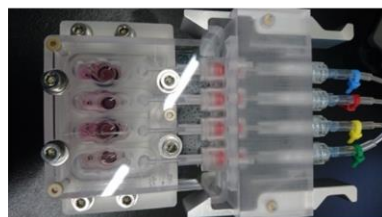
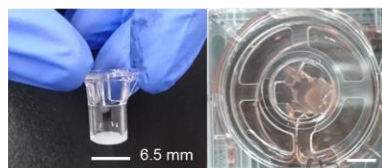
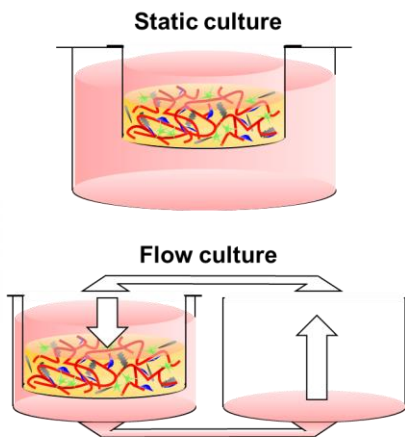


D-Mannitol 250 mg.mL<sup>-1</sup>

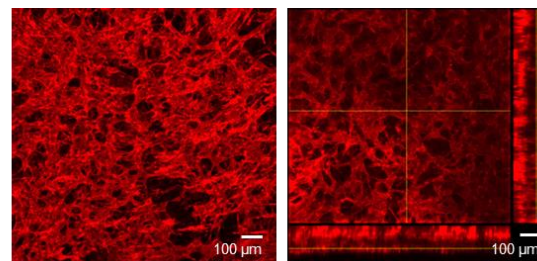


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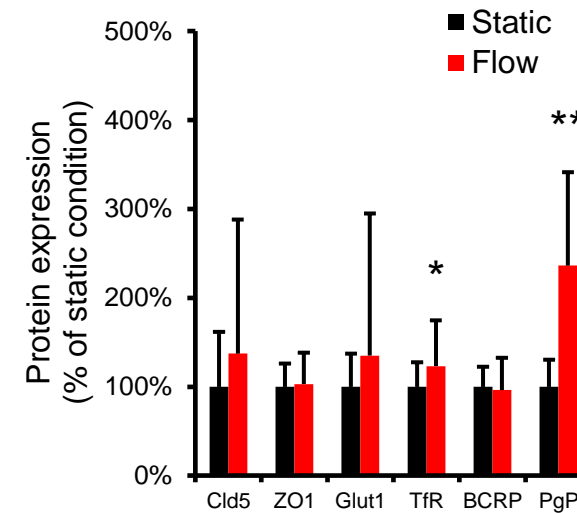
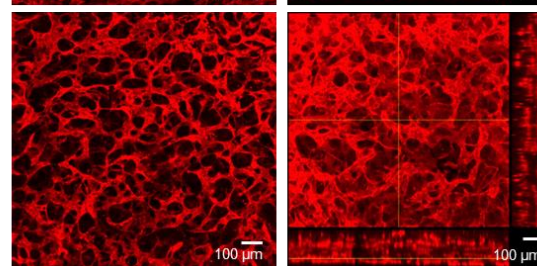
## First assays under medium flow



Static culture



Flow culture



## II. DEVELOPMENT OF A VASCULARIZED GBM TUMOR-ON-CHIP

*Work in progress*



Sylvain Chamouton  
Project ISIFC 2022



Marion Pouit  
Intern ISIFC 2023



Roumaïssa Mosbah  
Intern M2 2023

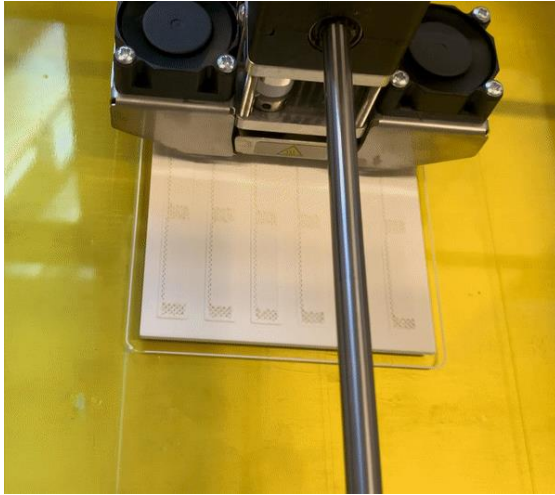


Léo Durand  
Project ISIFC 2023



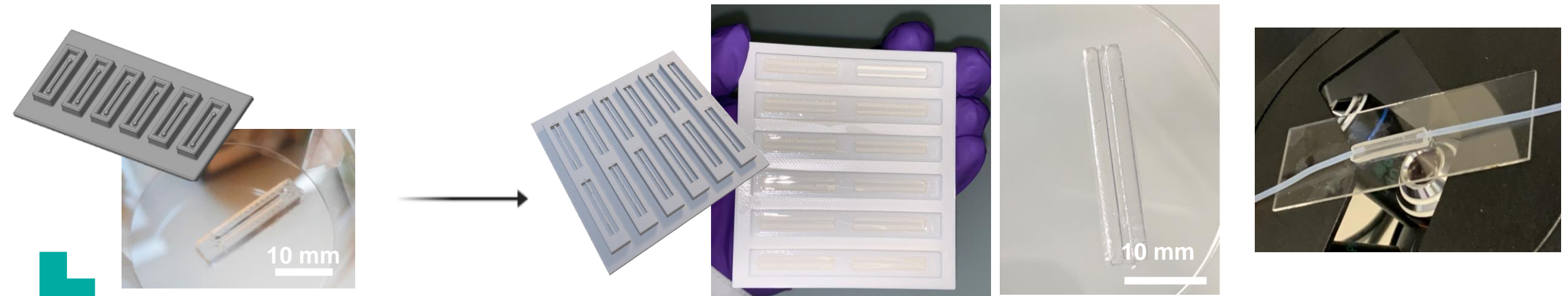
Maxime Gourgues  
PhD student 2023-2026

# MICROCHIP DESIGN



4<sup>th</sup> prototypes:

- Mold obtained by CAD and PLA (polylactic acid) 3D printing
- PDMS (polydimethylsiloxane) cast and polymerized
  - Common because cheap, easy to handle, permeable to gaz
  - + or – optically transparent
  - Planned functionalization to avoid drug adsorption

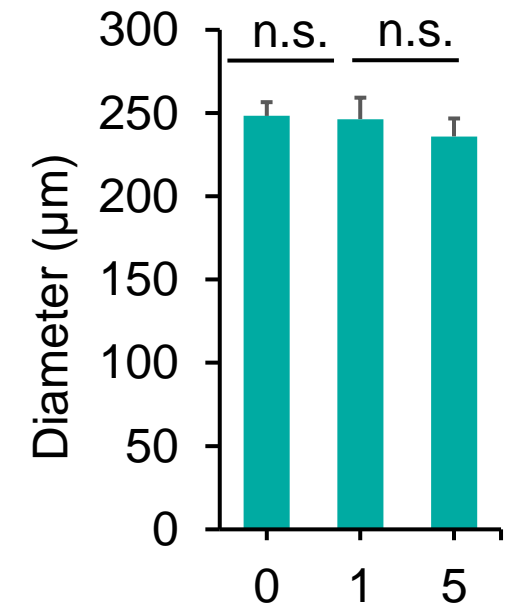
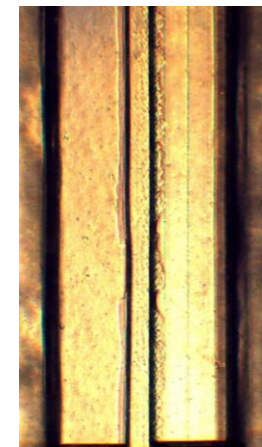
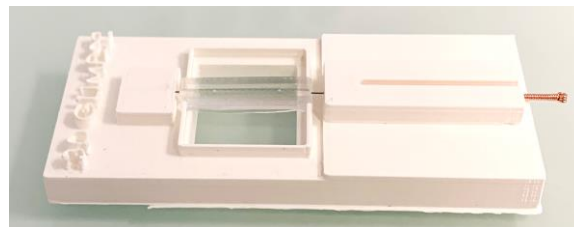
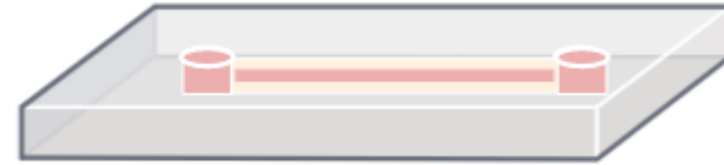




# CENTRAL CHANNEL

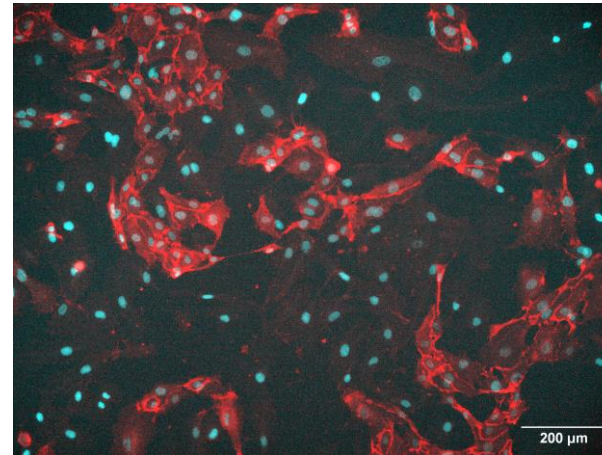
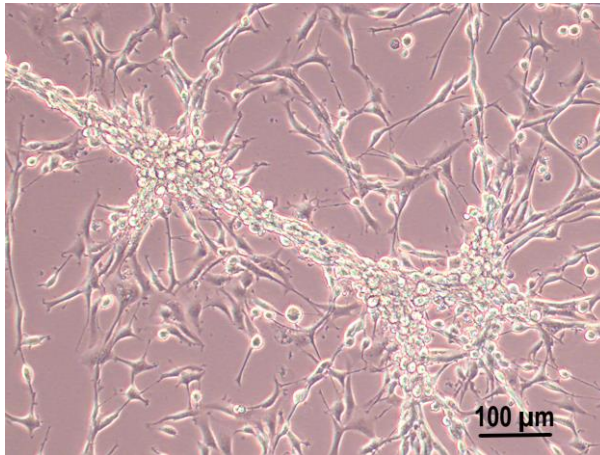
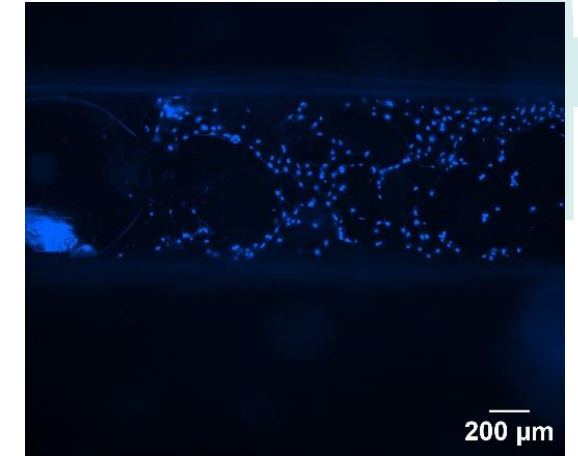
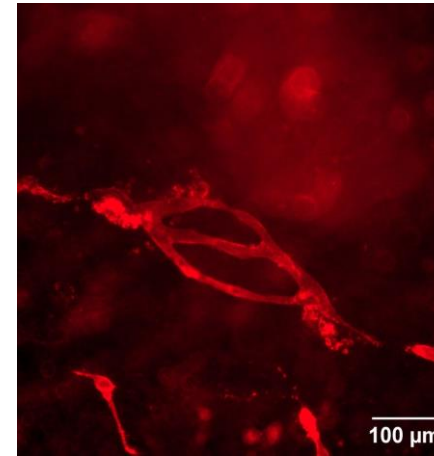
Veinule as a central channel

- Carved with an acupuncture needle
  - Perfused with medium to mimic blood flow
- 
- ✓ Prototypes of the device needed to guide the needle
  - ✓ The diameter of the veinule in the hydrogel is stable
  - Complexification of the veinule trajectory
- Impact of the veinule design on angiogenesis



# CELL COCULTURE

- Reproduction of the vasculature self-organization conditions
- Optimization of the collagen-fibrin hydrogel
- Endothelial cell seeding in the central veinule

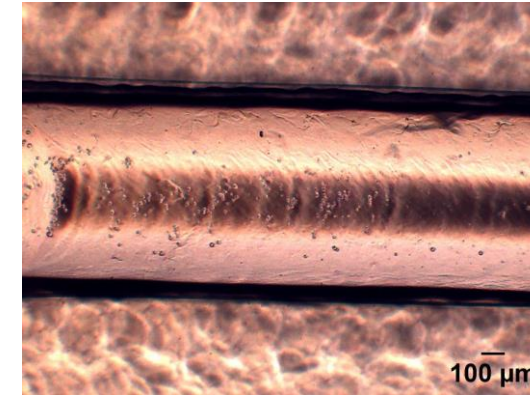
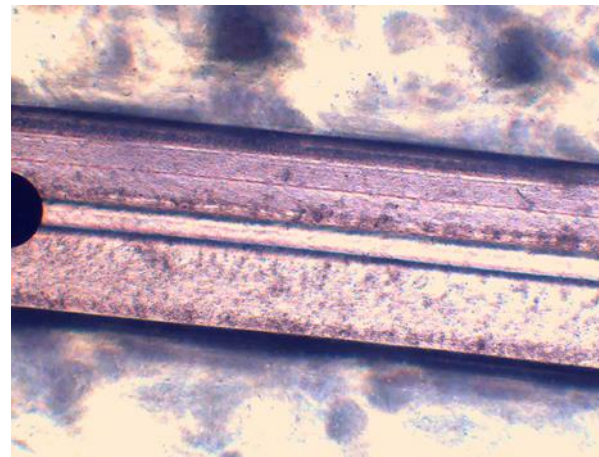
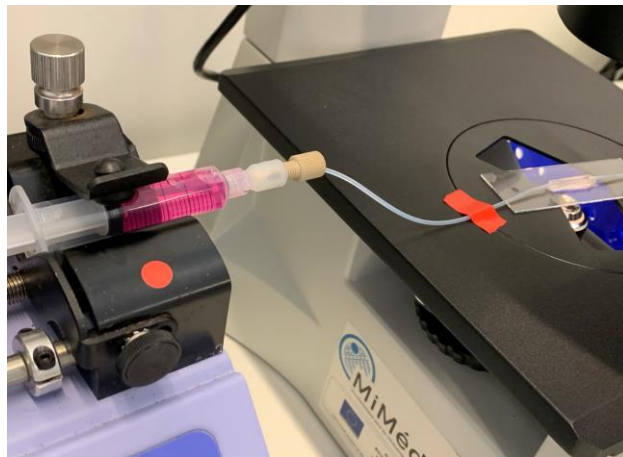


- Optimization of the coculture with U87-MG glioblastoma cell line  
→ Study of the 3D organization, and angiogenesis in a tumoral environment

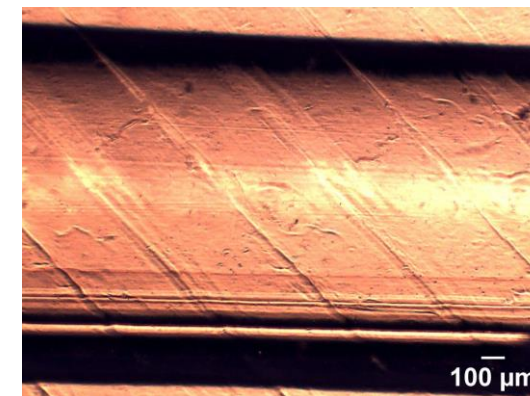
# FLOW

- ☑ First assays in acellular condition
- ☐ Optimization of the chip design
- ☐ Optimization of the flow parameters
  - speed, volume, duration
  - pump type and controllers

→ Impact on the tissues and cell phenotypes

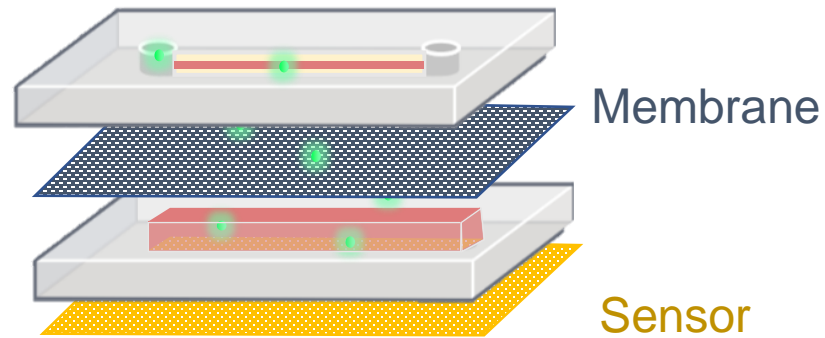


Open chip

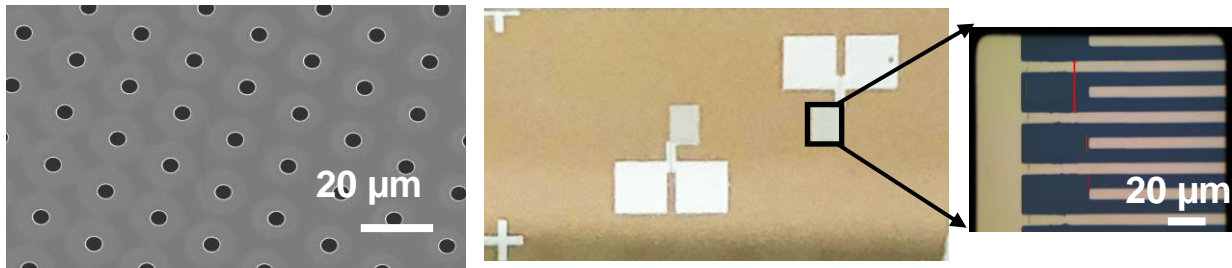


Close chip  
in PDMS

# BIOSENSORS



- Transport biosensor by acoustic waves
- Controlled microporous membrane for cell culture
- Other physical sensors (pH, T, O<sub>2</sub>)



Muhammad Hamidullah

Postdoctoral MSCA SmooC

# COLLABORATIONS

**Osaka University**  
 Chemical engineering department  
**Toppan Joint venture**



**Tokyo University of Pharmacy  
 and Life Science**



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**Université de Franche-Comté**



**BaRITOn UMR 1053**  
**Université de Bordeaux**



Michiya MATSUSAKI	BBB model, extracellular matrix
Asuka YAMADA	Collagen microfiber sponge
Tomomi FURIHATA	BBB cell lines

Arnaud BEDUNEAU	Nanocarriers against glioblastoma
Stéphane ROUX	

Océane MARTIN	Microbiology, oncology Gut-Brain axis
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# LOCAL TEAM



<b>Agathe FIGAROL</b>	<b>Nano-bio-interaction, tissue engineering</b>
Thérèse LEBLOIS	Biomicrodevices and biosensors
Vincent HUMBLLOT	Biointerfaces, chemical functionalization
Franck CHOLLET	Microfabrication, electroacoustic
Muhammad HAMIDULLAH	Microsystems, biosensors
Bruno WACOGNE	Optical sensors
<i>Maxime GOURGUES</i>	PhD student, GBM-sur-puce
<b><i>Upcoming</i></b>	<b><i>M2 intern</i></b>
<b><i>Upcoming</i></b>	<b><i>PhD student</i></b>
Céline ELIE-CAILLE	Microvesicles, exosome

# FUNDINGS



fondation  
DESCROIX-VERNIER



UNIVERSITÉ DE  
FRANCHE-COMTÉ



SPIM  
école doctorale sciences pour l'ingénieur et microtechniques



THANK YOU FOR YOUR ATTENTION