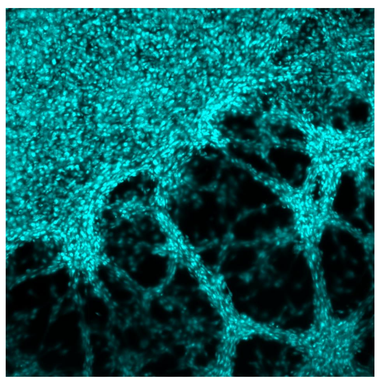
Development of an instrumented physiological microdevice for the modelisation of human brain vasculature in tumoral context

Maxime Gourgues[1], Roumaïssah Mosbah[1], Marion Pouit[1], Thomas Sivier[1], Agathe Figarol[1], Vincent Humblot[1]



Immunofluorescence view of tumor cells U87-MG, x10 magnification

Introduction

Glioblastoma is the most frequent and aggressive **brain tumor**. Conventional treatments are not sufficiently effective, and the development of new drugs can be hampered due to the **blood-brain-barrier (BBB).** For faster drug screening, current studies aim at reproducing *in vitro* the cerebral vascular microenvironment, in a healthy or tumoral context, but often neglect the impacts of extra-cellular matrix (ECM) or flow conditions.

This study focuses on the design of a microchip hosting a hydrogel mimicking the **extra-cellular matrix (ECM),** within which a **cell coculture** could organize as a **vascular network**. This network will be perfused to reproduce the blood flow.

3D printing

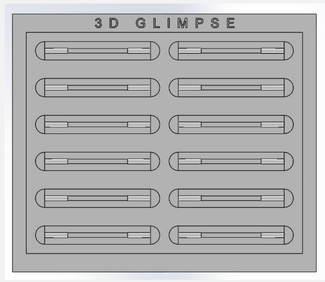
- CAD software (SolidWorks) design of PLA (polylactic acid) mold
- **PDMS** (polydimethylsiloxane, Sylgard 184) casting
- Reticulation of PDMS, at 80°C for 2h

→ Bending of the PLA mold (thermal retractation)
- Other materials could have higher thermostability, but lesser biocompatibility
- Infill patterns with increased mechanical did not change the retractation
- New reticulation parameters of **40°C during 8h**

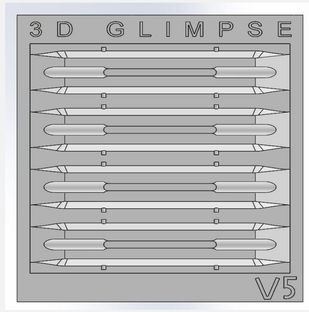
→ Residual stickiness, lack of PDMS thermal retractation
- Optimization in chip design to ease demolding

→ Printing marks that hampered microscopic observations
- Finer 3D printing nozzle,
- Adjustments of printing speed and layer height

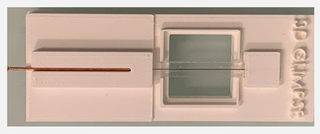
→ Previous chip versions could not withstand tubing and glass slide enclosing
- Junction diameter is set at 1,4 mm diameter, taking into account **PDMS retractation to ensure sealing**



V1 CAD mold for casting PDMS chips



V5 CAD mold



2 cm long chip with needle insertion guide

Hydrogel and needle

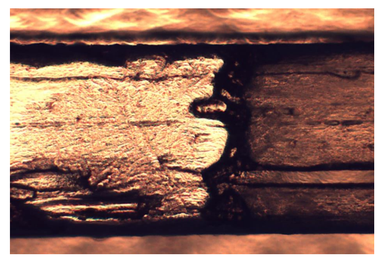
Hydrogel composition:
- **Fibrinogen** (6,6 mg/mL)
- **Thrombin** (10 µL/mL)
- Type-I microfibrillar **collagen** (16 mg/mL)

Dispersed in **medium culture** with cells for 20 µL total volume to fill the chip channel

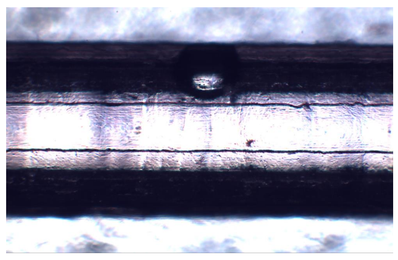
A needle is introduced to form a channel in which **endothelial cells** (HBEC-5i) will be seeded to mimic a venule

→ Needle placement
- 3D-printed guide ensure proper needle withdrawal after gelation

→ Too fast gelation
- Chip cooling to slow down reaction thermodynamics was not enough.
- Choice was made to set medium culture at 4°C, allowing to repeatedly cast one chip before gelation
- But putting pressure on cell viability



Fast gelation issues hence uprooting of hydrogel



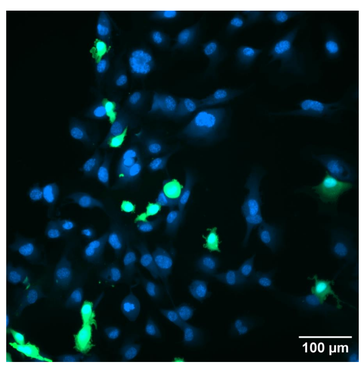
Hydrogel 7h after needle pullout

Cell coculture

Three other human cell types were used
- **Pericytes** (HP) have a major angiogenic role
- **Astrocytes** (HA) reinforce the newly created vasculature
- **Glioblastoma** tumoral cells (U87-MG) may enable the angiogenesis but also disturb the microenvironment

2D tests allowed to determine optimal cell and medium proportions
- No sign of cell lack of confluence with any other of the four medium types
- HP growth is not hampered by U87 presence

The coculture **HBEC:HA:HP was set at 1:0,5:2 ratio,** with U87 various ratio modelling different tumor stages

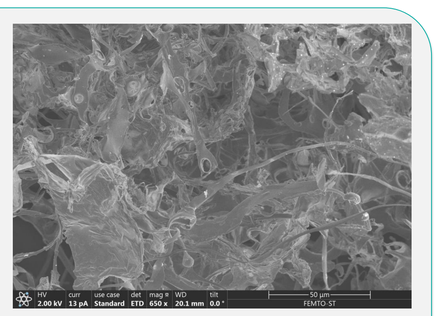


U87 (Cell Tracker green) and HP (Hoechst blue)

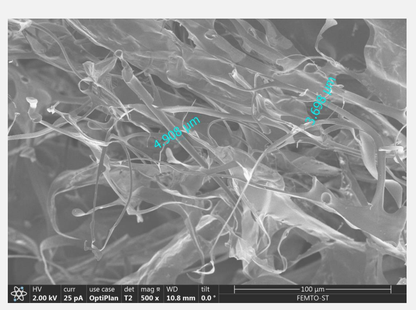
SEM observation

Further works on the collagen microfibers is undergoing to understand the effects of conformational changes, such as thermal crosslinking and partial denaturation by heating at 200°C for 24h under vacuum (50 mbar)

Here a scanning electron microscope (SEM) view of collagen that has been:
- **Homogenized**
- **Sonicated** at 100W for 50 min to shorten fibrils medium length, promoting formation of scaffold-like structures and seemingly collagen dispersion
- **Freeze-dryed** at 80°C, unveiling medium diameter of collagen fibrils of 2-10 µm



No sonication, x650 magnification



After sonication, x500 magnification

Conclusion

CAD mold was designed for PDMS chips fabrication. ECM composition and stiffness, enhancing angiogenesis and cells adhesion, is optimized through physical treatments of collagen, and changes in its conformation is observed. Along with cell coculture tests, all those steps get use closer to the **development a 3D BBB capillary system**, with human cells embedded in a collagen-based ECM cast into PDMS chips.

Perspectives

The next steps will consist in setting **device under flow conditions** at a flow rate lower than 100 µL/min, with the use of a peristaltic pump and plugging into a microfluidic system, **modelling the blood flow** and its effect on the BBB.

The incorporation of different cell types into the hydrogel will allow us to bring modelisation of human brain microvascular environment closer.

Contact : Gourgues Maxime
Tel. : (+33) 3 81 66 61 62
E-mail : maxime.gourgues@femto-st.fr

1: FEMTO-ST Institute, UMR CNRS 6174, UBFC
15B Avenue des Montboucons, Besançon
https://www.femto-st.fr