

GlioLight Workshop

1st-2nd September 2026

VINČA Institute of Nuclear Sciences, Belgrade, Serbia

***Experimental in vitro model systems
for tumor biology studies and therapy development***

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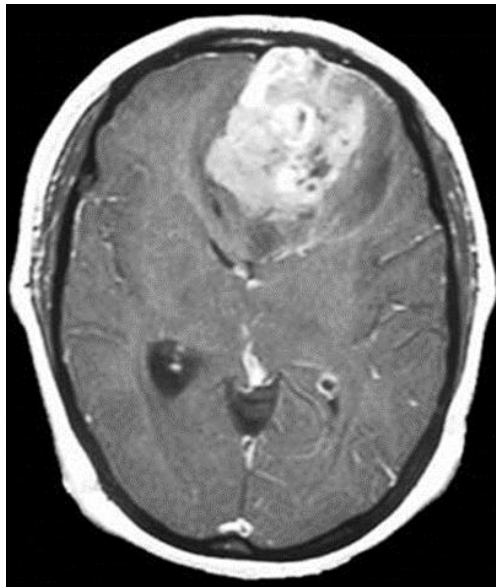
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Experimental models

The tumor mass

Limits in nutrients
Low oxygen ($\leq 1\%$)
Connected cells



wikipedia

Experimental conditions

***in vitro* / Cell culture**

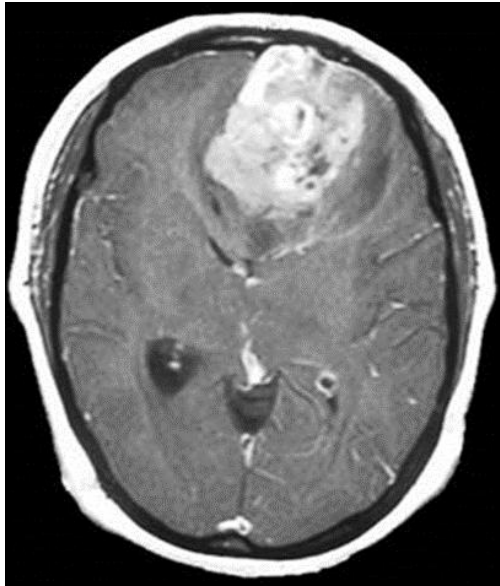
5-10% FCS, high glucose
21% oxygen
Disconnected cells

***in vivo* / Animal model**

Physiological gradients in nutrients
Oxygen gradient
Connected cells

Species / immune incompatibilities

Experimental frame: clinically-relevant *in vitro* model



Limits in nutrients

Use low glucose conditions

Low oxygen ($\leq 1\%$)

Use low oxygen conditions

Tumor = 1-3 % oxygen

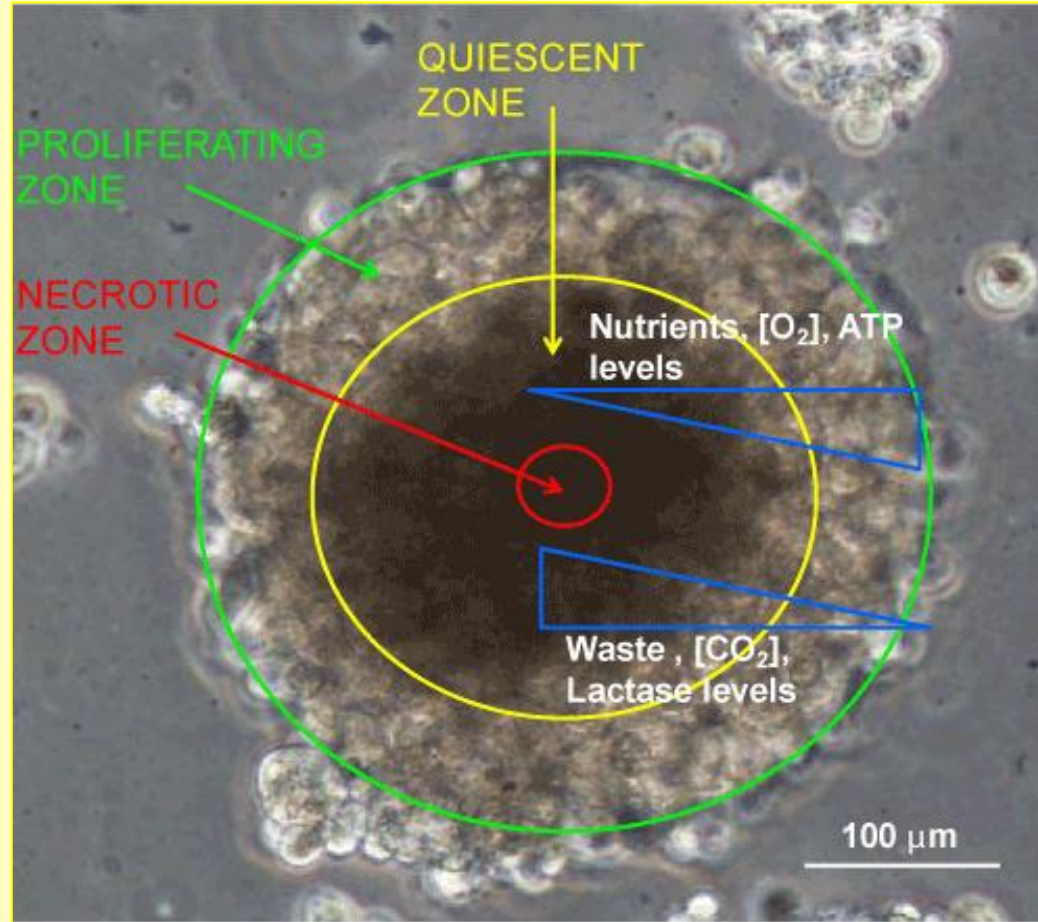
Brain = approx. 5% oxygen

Connected cells

Use 3D cultures instead of 2D cultures

3D culture - Tumor spheroids mirror the tumor mass

- Limits in nutrients
- Oxygen gradient
- Connected cells
- ✓ 3D architecture of tumors
- ✓ distribution /diffusion
- ✓ cell-cell interaction
- ✓ cell-ECM interaction
- ✓ multicellularity

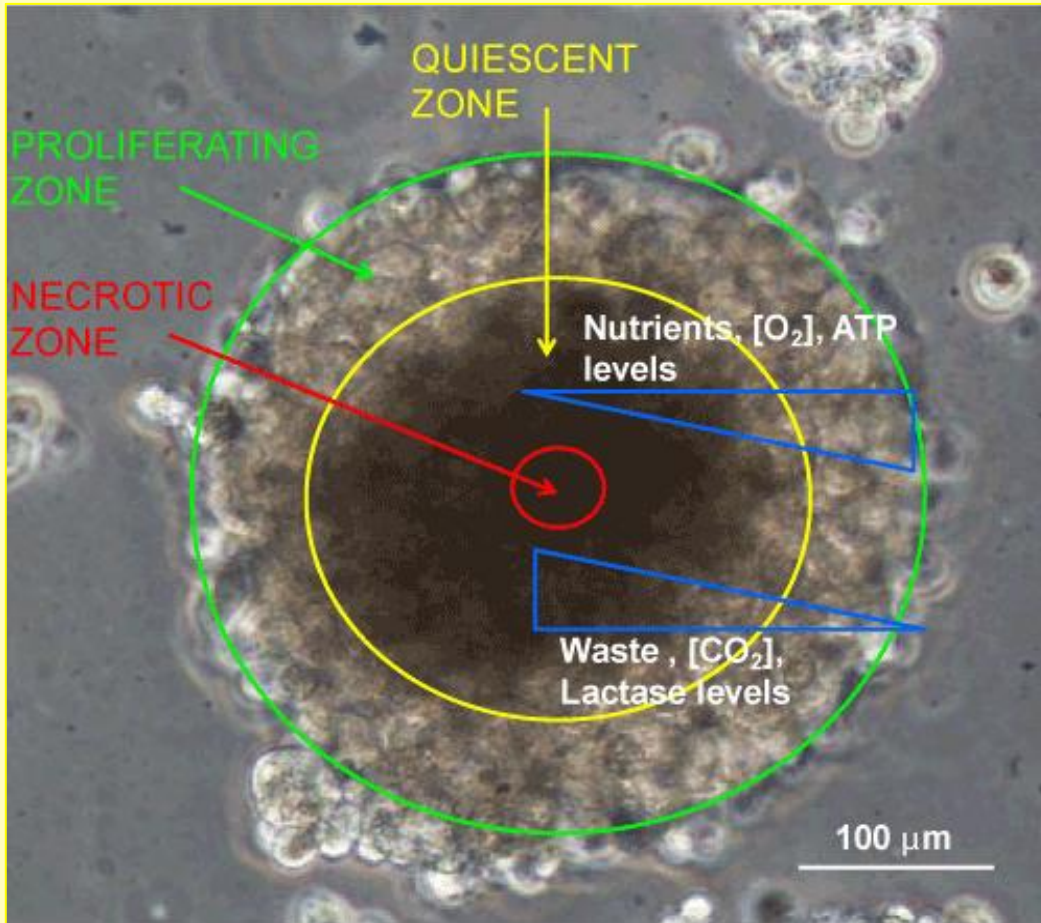


http://vbl.ts.infn.it/SiteVBL/projectSpheroid_files/velocityfield.jpg

Spheroid in suspension

Spheroid in a matrix / gel

„3D culture“ – Tumor spheroids mirror the tumor mass



http://vbl.ts.infn.it/SiteVBL/projectSpheroid_files/velocityfield.jpg

Limitations/challenges

❖ Tumor mass:

Complexity of its cellular composition:

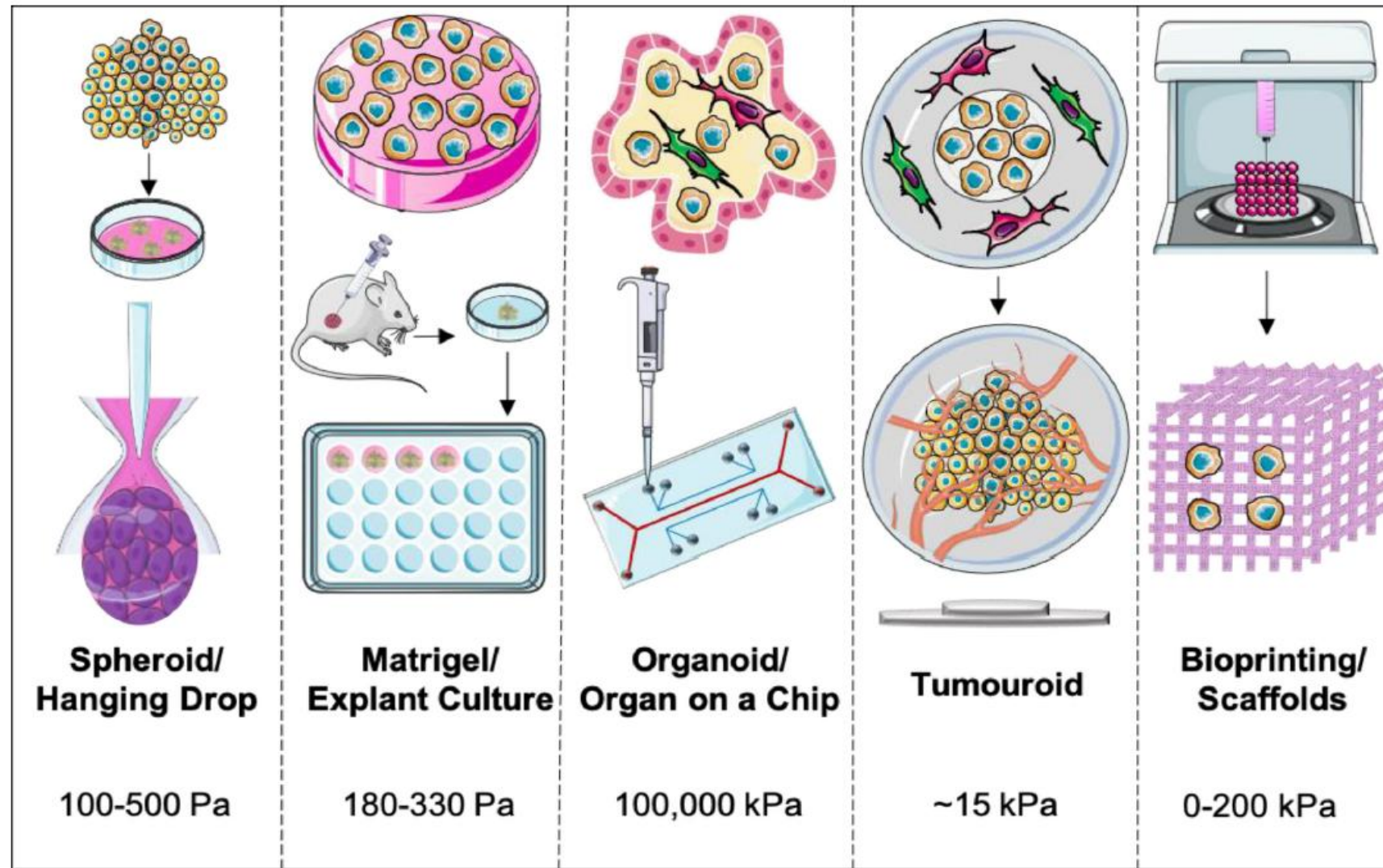
Tumor cells, Tumor-stem like cells, Glial cells,
Endothelial cells, Immune cells....

❖ Surrounding tissue: the extracellular matrix:

Molecular and cellular complexity
Viscosity, elasticity

❖ Vascularization of the tumor

3D cancer models



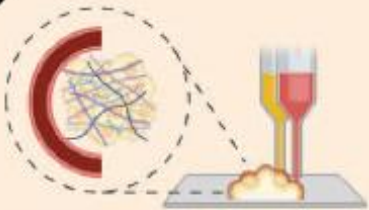
„Emerging“ 3D models

Spheroid with
Patient-derived cells

Organoid with
iPSC-derived cells

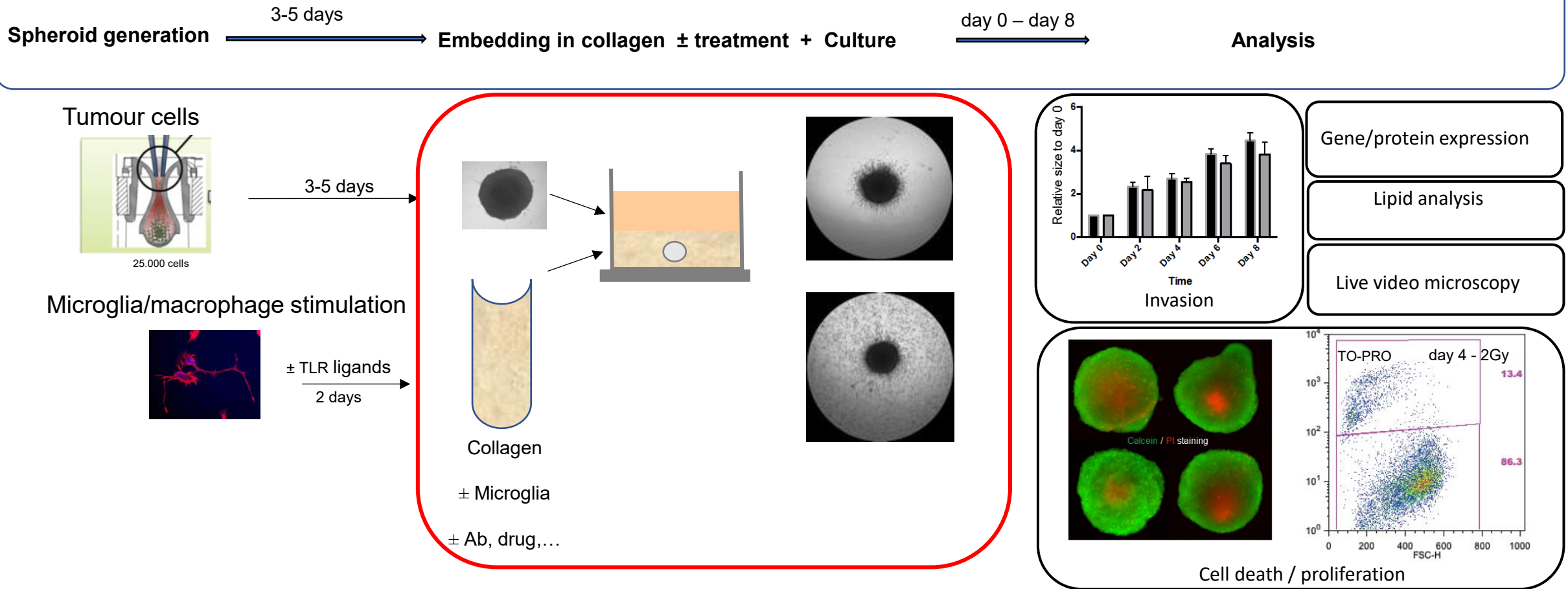
Fusion
spheroid/organoid

Bio-printing

		Methods	Pros and Cons
Patient-derived	A 	• Direct culture of minced tumor • Use agitation and/or Matrigel to support 3D structure	Biologically-representative, high fidelity Gradual loss of other cells, heterogeneous organoids
iPSC-derived	B 	• Neoplastic transformation of iPSC-derived brain organoids • Brain organoids with patient mutations	Defined genetics, reproducible and high-throughput system Lack genetic heterogeneity, neurodevelopmental model
Fusion	C 	• Fusion of glioblastoma cells/spheres/organoids with brain organoids	Human neural substrate, convenient toolkit Neurodevelopmental model
Bio-printing	D 	• Encapsulation of tumor cells, ECM and other cell types via bio-printing on microfluidic chips	Flexible components with ECM and other cell types, Vasculature and BBB reconstruction Difficult to maintain, lack environment specificity

Example from our laboratory (1)

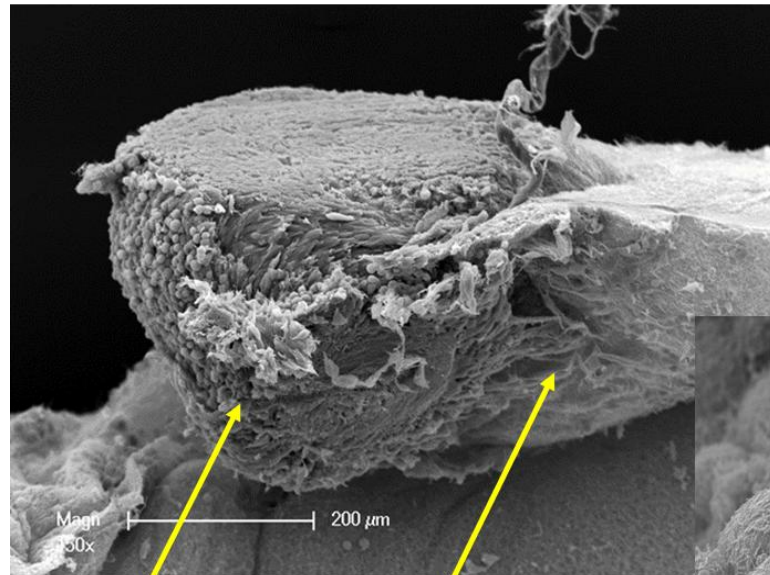
Spheroids generated with established tumor cell lines and primary microglia or macrophages



Versatile system : Spheroid in suspension versus embedded spheroid
Addition of reagents or cells in the matrix /medium

Morphology of the spheroids

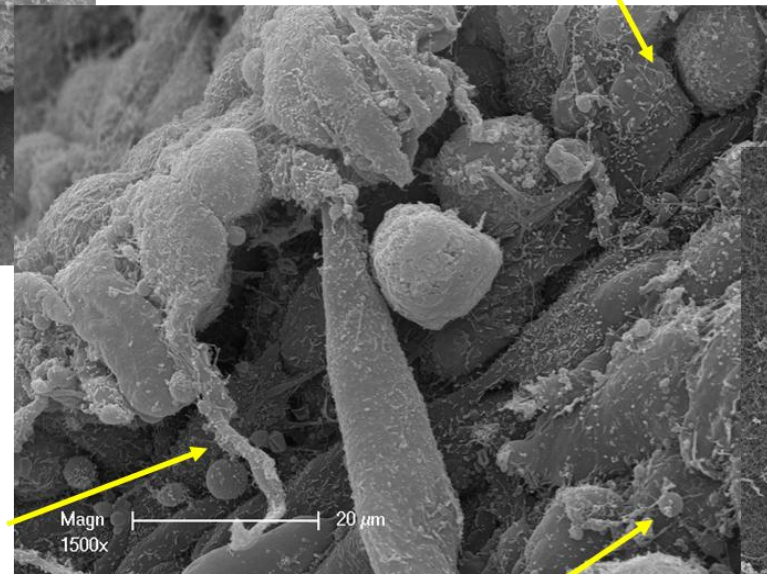
Scanning Electron Microscopy



Spheroid

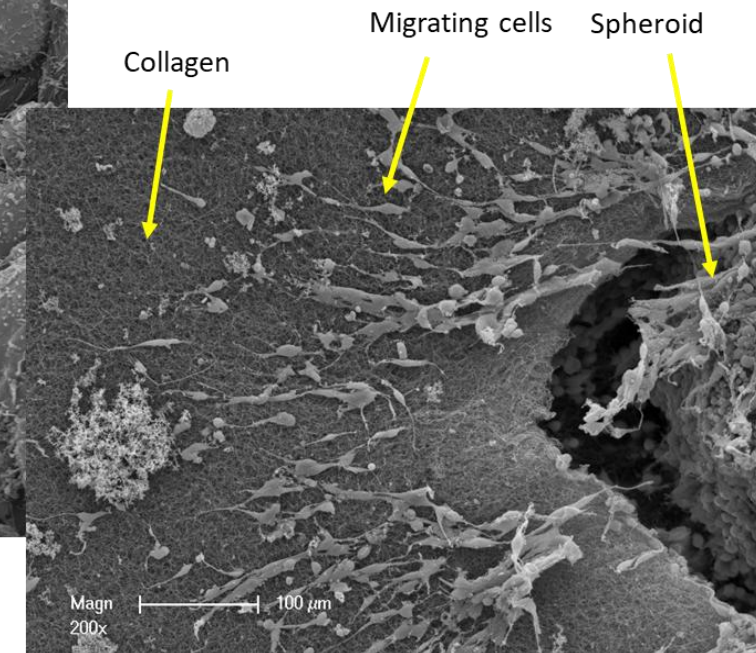
Cells extending in collagen

Microtube?



Blebs

Microvilli

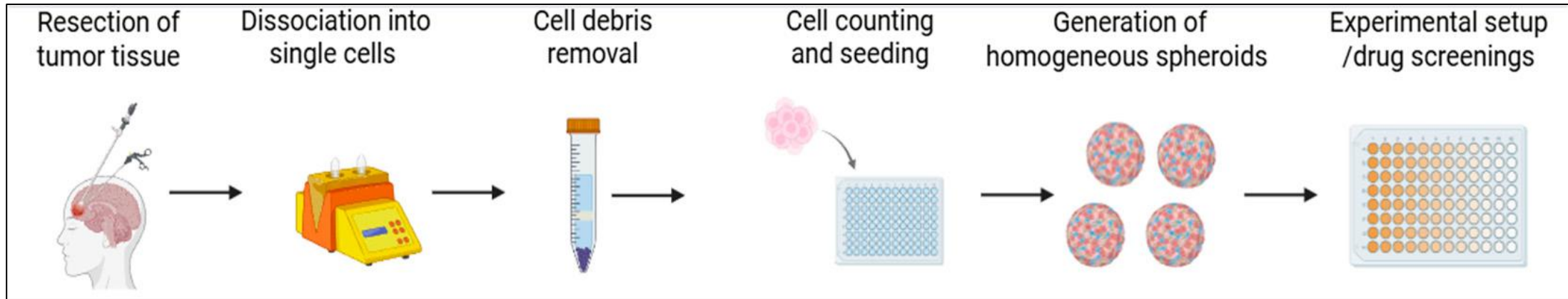


Collagen

Migrating cells

Spheroid

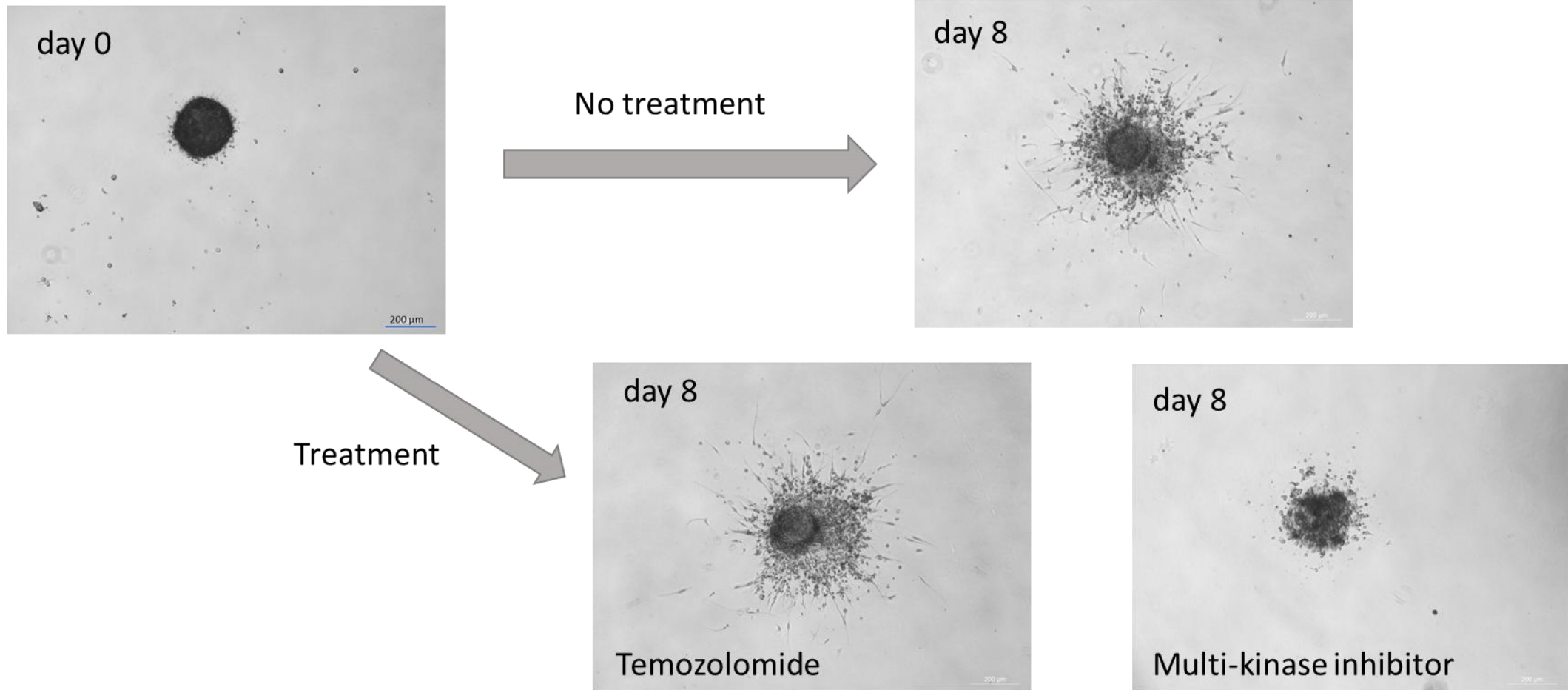
Example from our laboratory (2): Primary patient-derived tumor cells spheroids / tumoroids



Aim = Ensure the generation of an homogenous set of spheroids:

- representing a screening platform for drugs (survival/death)
- but also a tool for studying cells in 3D in response to various treatments (localisation, migration; gene/protein expression, etc)

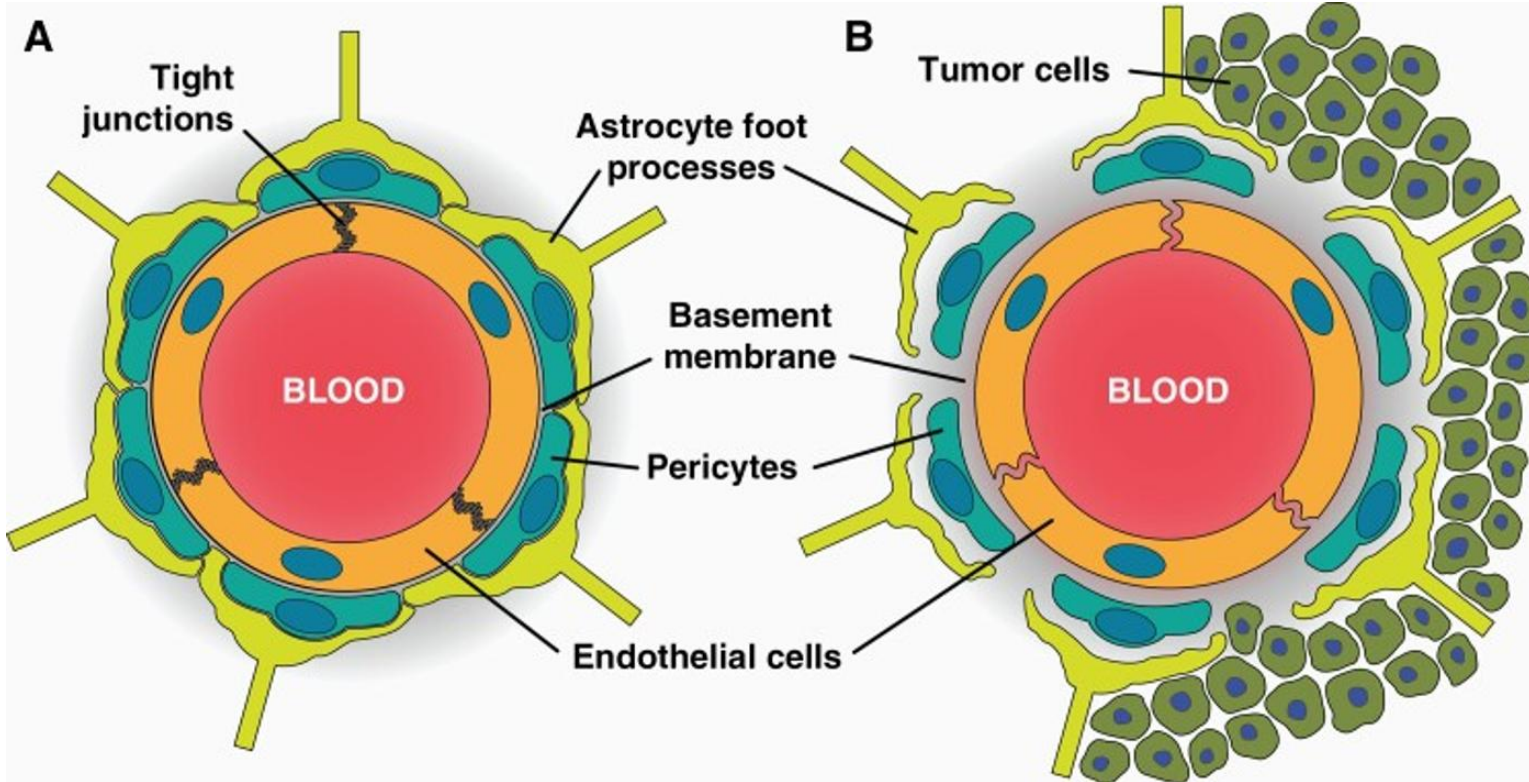
Spheroids of primary human glioblastoma cells: A screening platform for testing drugs on invasion capacities



A missing element in 3D models: blood vessels

Blood Brain Barrier

Blood Tumor Barrier



Project with Prof. Marijana Petkovic
(Application to DAAD)

Models of human 3D tumoroids
integrating blood–tumor barrier
for predictive testing of
photodynamic and combination
therapies (IGNITE)

Self assembling spheroids with human cells

ARTICLE

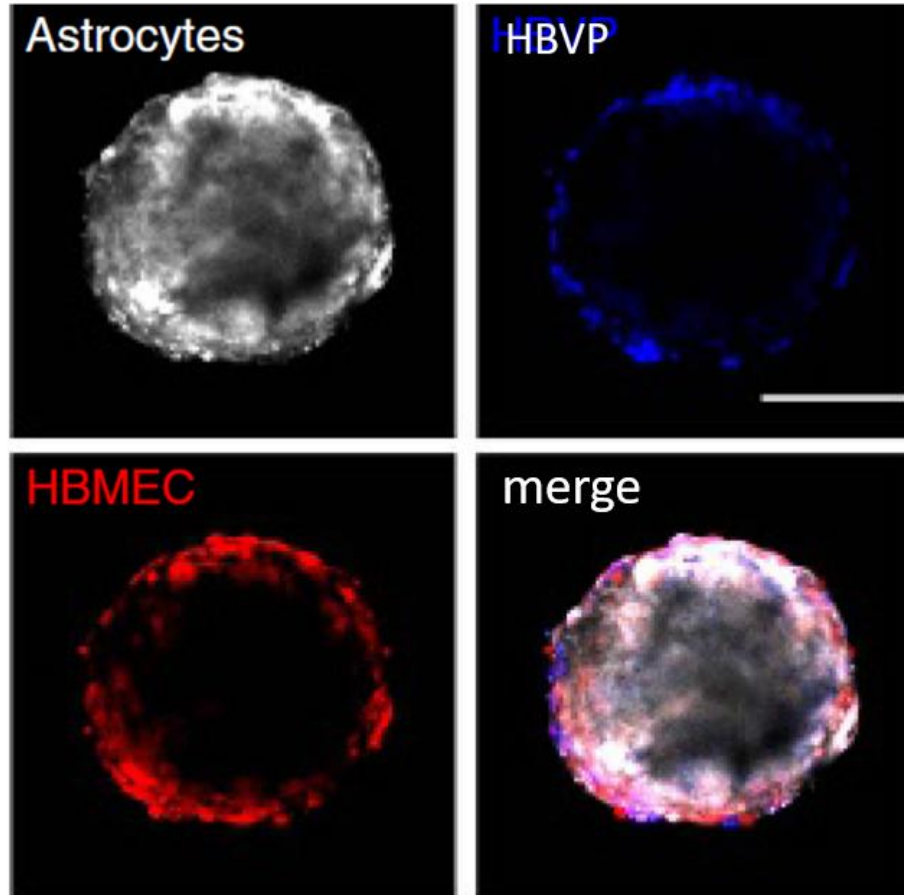
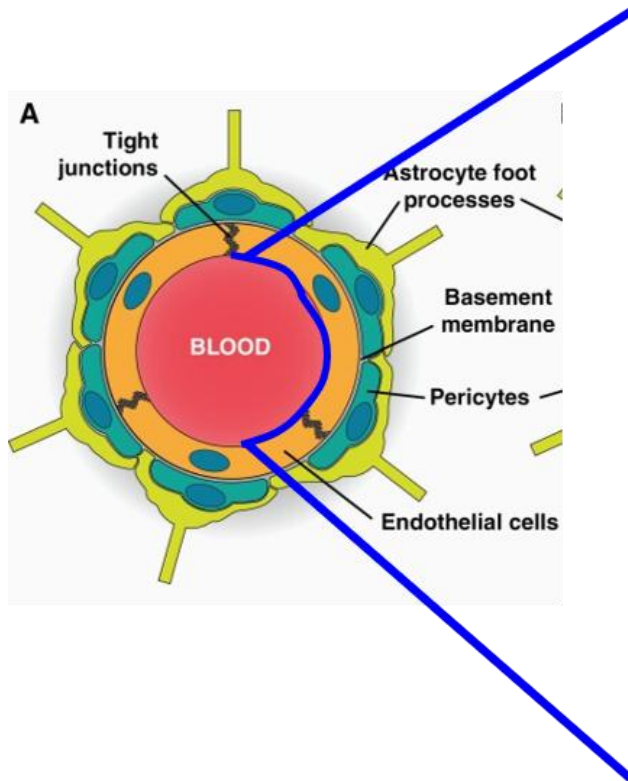
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OPEN

Blood-brain-barrier spheroids as an *in vitro* screening platform for brain-penetrating agents

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- Core:
Astrocytes
- Surface:
Brain microvascular endothelial cells (HBMEC)
Brain vascular pericytes (HBVP)

Conclusion: in search of the „ideal“ model

- Cellular complexity of the tumour
- Extracellular matrix complexity
(cells, molecules, viscoelasticity)
- Tumor environment
(e.g. brain cells, vessels)

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