Innovative 3D microfluidic brain tumor-on-a-chip systems: design, characterization, and preliminary testing with chemotherapy drug-loaded nanocarriers

Attilio Marino[1]
Matteo Battaglini[1]
Omar Tricinci[2]
Alessio Carmignani[1]
Gianni Ciofani[1]

[1]Istituto Italiano di Tecnologia, Smart Bio-Interfaces, Viale Rinaldo Piaggio 34, 56025 Pontedera
[1]Istituto Italiano di Tecnologia, Center for Materials Interfaces, Viale Rinaldo Piaggio 34, 56025 Pontedera

attilio.marino@iit.it

Central nervous system (CNS) tumors comprise over 100 histologically distinct subtypes and are among the most fatal cancers [1]. In children and adolescents, they represent the second cause of death (26% of cases) after leukemia (28%) [2]. Different innovative and personalized anticancer drugs, even if potentially able to treat brain tumors, have not been reached or passed the clinical trial phases due to their limited ability to reach the brain *via* systemic administration [3]. The blood-brain barrier (BBB; *i.e.*, the biological barrier characterized by vascular endothelial cells tightly connected to neurons, astrocytes, and pericytes) represents the main obstacle to the drug delivery from blood microcapillaries to the brain parenchyma. Therefore, intensive research activities have been applied in the last 10 years to the realization of *in vitro* platforms realistically reproducing the BBB to investigate drug delivery to the brain.

In this scenario, our research group designed and characterized the first 1.1 scale model of the brain microcapillaries mimicking blood flow through a fluidic system. The faithful reconstruction of the brain microcapillaries has been obtained through two-photon lithography, an innovative microfabrication approach allowing for rapid and high-resolution prototyping of 3D microstructures (Figure 1). The proposed model can be implemented in at least two configurations. A first device, simple and user-friendly, allows the single-step seeding of endothelial cells and astrocytes on the external surface of the porous micro-tubes. A second one, more biomimetic but more complex, allows the integration of multiple cell types associated with the BBB in physio/pathological conditions (*e.g.*, endothelial cells, astrocytes, pericytes, neurons, and tumor cells). In both configurations, the obtained barrier shows good performances in terms of trans-endothelial electrical resistance (TEER) and reduced molecular permeability. The smart integration of the cell cultures to the microfluidic chip can be provided by our patented solution, which allows for static magnetic field (SMF)-assisted docking and automatic assembly of the magnetized scaffolds (Figure 2). The resulting brain tumor-on-a-chip systems allowed our group to test the anticancer effectiveness of nutlin-3a and nutlin-3a-loaded lipid nanocarriers. Finally, thanks to the complexity of our multicellular model, the specificity of the anticancer effect of nutlin-3a toward glioblastoma cells compared to neural and endothelial cells has been demonstrated.

The proposed brain tumor-on-a-chip systems promise to close the gap between *in vitro* and *in vivo* testing with a pivotal impact on drug discovery. Indeed, the proposed devices will provide more reliable and repeatable results *in vitro*, thus significantly contributing to lowering the risk of both early- and late-stage failures of drug testing in clinical trials. Also, these *in vitro* platforms might produce ethical benefits by excluding drug candidates with low BBB crossing capabilities from *in vivo* tests and therefore reducing animal experimentation.

References

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Figures

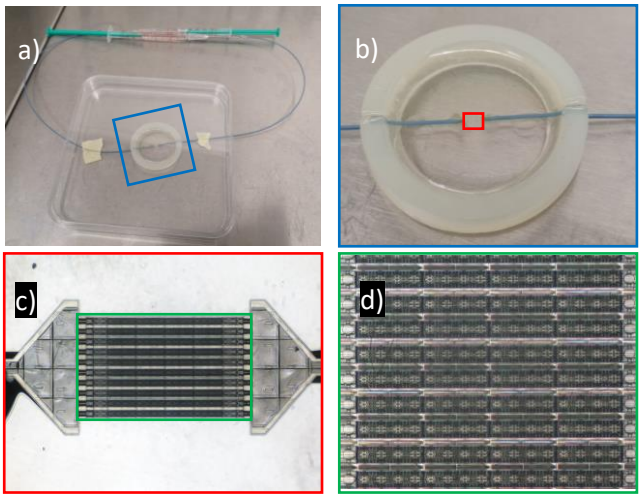

Figure 1. 3D microfluidic BBB system. a,b) System connected to syringes. c,d) Microscope images of the porous microtubes in parallel. Endothelial cells can be seeded inside or outside the microtubes to mimic blood microcapillaries.

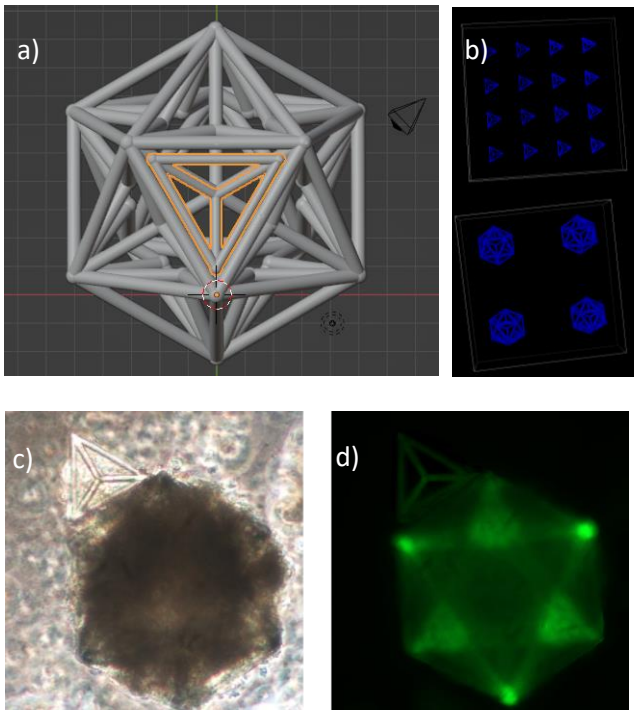

Figure 2. Magnetic self-assembly of cell co-cultures. a) 3D rendering of the great dodecahedron with the tetrahedron (highlighted in orange). b) 3D confocal laser scanning microscopy imaging of the tetrahedrons (top) and great dodecahedrons (bottom) fabricated by two-photon lithography. c,d) Magnetic self-assembly of the U-87 glioblastoma cells in the great dodecahedron and HCMEC endothelial cells in the tetrahedrons.