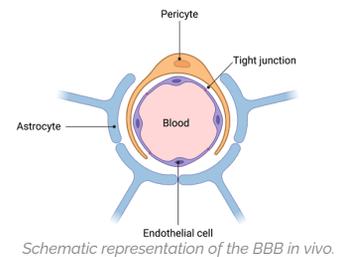


Blood-Brain Barrier (BBB) Antibody Penetration Assay on Gri3D®

Introducing BBB on Gri3D®

Gri3D® is a ready-to-use platform for high-throughput and reproducible organoid culture. Here we describe its use to scale up the generation of highly reproducible blood-brain barrier (BBB) organoids. Up to 55 organoids self-organise to a BBB mimicking architecture in a single well. Antibody penetration is evaluated *in situ* via fluorescence imaging in a fully automated workflow.

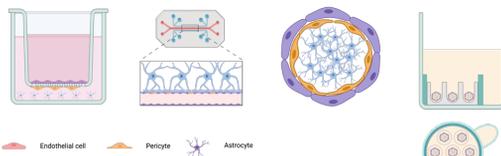


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BBB models: state of the art

Multicellular BBB models are comprised of brain endothelial cells, supported by astrocytes and pericytes. Commonly used models are constructed using transwell inserts and microfluidic devices or rely on cellular self-aggregation as is the case for BBB organoids [1]. The latter model allows direct cell-cell interactions and recapitulates key cellular and molecular properties of the BBB, but has limitations in terms of scale, ease of use and analysis [2]. The use of Gri3D® overcomes these challenges.

Figures created with BioRender.com



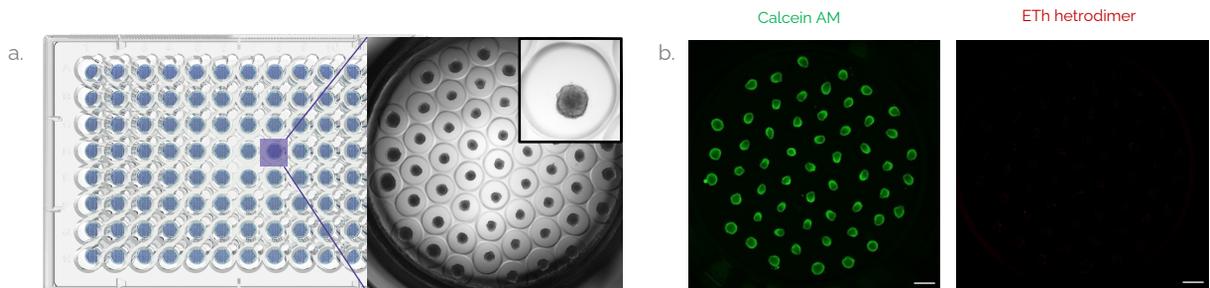
Schematic representation of in vitro BBB models.

| | Transwell | Microfluidic | Cell Aggregation | Gri3D® |
|--------------------------|-----------|--------------|------------------|--------|
| Data points/plate | ■ ■ | ■ | ■ ■ | ■ ■ ■ |
| Ease of seeding | ■ | ■ | ■ ■ ■ | ■ ■ ■ |
| Ease of assay | ■ | ■ | ■ ■ | ■ ■ ■ |
| Presence of shear stress | ■ | ■ ■ ■ | ■ | ■ |
| Cell-cell contact | ■ | ■ ■ | ■ ■ ■ | ■ ■ ■ |

Comparative table of in vitro BBB models. Adapted from [2,3]

BBB organoids on Gri3D®

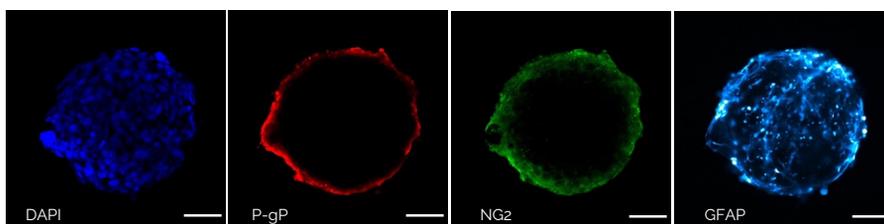
Gri3D® 96WP imaging-bottom 600 µm microwells are used to scale up the generation of multicellular BBB organoids. Endothelial cells, pericytes and astrocytes are seeded simultaneously and self-assemble within 48 hours maintaining high viability.



BBB organoids grown on Gri3D® 96WP imaging-bottom 600 µm. **a.** Brightfield image showing one well with 55 BBB organoids; **b.** Live/dead staining of BBB organoids. Scale bars: 600 µm.

In situ immunofluorescence

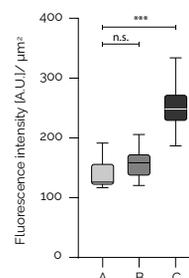
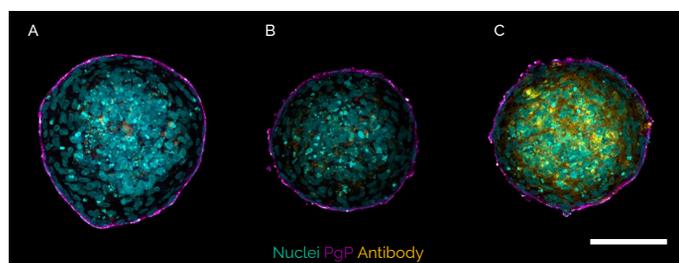
The different cell types self-assemble in layers, with endothelial cells surrounding the pericytes that encapsulate a core of astrocytes. The presence of specific markers is revealed directly on Gri3D® by immunofluorescence.



Immunofluorescence of different cell types: endothelial cells (P-gp), pericytes (NG2) and astrocytes (GFAP). Scale bars: 50 μ m.

Antibody penetration assay

After organoid assembly, cells are incubated with the antibodies of interest, fixed and immunostained on Gri3D® following an automatable protocol. Antibody penetration is assessed by indirect immunofluorescence and imaged with a confocal microscope.



Immunofluorescence and quantification of different antibody constructs penetrating BBB organoids: **A.** Untreated control; **B.** Non-targeting human IgG; **C.** Human brain shuttle. Brain endothelial cells are labelled with an anti-P-gp antibody. Scale bar: 100 μ m. Adapted from [4].

Highlights of the model

- **High throughput:** the use of Gri3D® 96 600 μ m microwells allows a **55 fold increase in organoid yield** compared to traditional suspension culture methods (more than 5000 datapoints in a single 96 well plate)
- **Fast: 10 fold decrease in plate cycle time of organoids** (seeding to read out) due to the integration of the entire workflow in situ on Gri3D®
- **Relevant:** BBB organoids closely represent the in vivo architecture and function of the BBB and are thus a suitable model for antibody penetration assays

How can we help you?

Antibody Penetration Assay Service

With the BBB model herein described, SUN bioscience assesses the penetration of antibodies and engineered antibody fragments by immunofluorescence *in vitro*.

Contact us for more information at enquiries@sunbioscience.ch

References

- [1] Cho, C. F. et al. Blood-brain-barrier spheroids as an in vitro screening platform for brain-penetrating agents. *Nat. Commun.* 8, 1–14 (2017) doi:10.1038/ncomms15623
- [2] Gastfriend, B. D., Palecek, S. P. & Shusta, E. V. Modeling the blood-brain barrier: Beyond the endothelial cells. *Curr. Opin. Biomed. Eng.* 6–12 (2018) doi:10.1016/j.cobme.2017.11.002
- [3] Bagchi, S. et al. In-vitro blood-brain barrier models for drug screening and permeation studies: An overview. *Drug Des. Devel. Ther.* 13, 3591–3605 (2019) doi: 10.2147/DDDT.S218708
- [4] Simonneau, C. et al. Investigating receptor-mediated antibody transcytosis using Blood-Brain Barrier organoid arrays. *bioRxiv* 1–25 (2021) doi.org/10.1101/2021.02.09.430382

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 Contact us
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 Address
EPFL Innovation Park
Bâtiment D
CH-1015 Lausanne