INFRAFRONTIER Complex *In Vitro* Models:

Blood Brain Barrier (BBB) organs-on-chip

Brief description:

GLUT1-BBB CIVM provides a standardized, high throughput, mouse stem cell-based (mESCs) organ-on-chip (OoC) model. By mimicking *in vivo* biological microenvironments, such as blood–brain interfaces and mechanical fluid flow, our model represents an *in vitro* 3D micro physiological system that reproduces functional units of mouse BBB.

The system is build on a 96 plate platform (modified from Akita®), containing 24 separate functional microfluidic BBB units (*figure 1*) and can be implemented up to 128 separate units in a 384 plate. Each unit consists of a microchannel, a culture chamber located between two standardised media reservoirs (inlet-outlet). Each culture chamber, composed of mESCs differentiated cells with the same genetic background, is divided horizontally into two parts by a thin membrane for endothelial barrier separating the lower culture chamber and open-top chamber for astrocyte/neuronal culture. Two addictional collection reservoirs, added at both sides of the chamber, allow the sampling of culture media after passage throughout the barrier, mimicking *in vivo* CSF. The BBB permeability of a plethora of labeled compounds can be easily evaluated in a scalable manner by measuring reservoir samples with a microplate reader.

Ad hoc designed experiments of fluorescent glucose BBB permeability between *in vitro* and *in vivo* GLUT1 mouse model return a validated high throughput *in vitro* model, through direct *in vitro/in vivo* comparison.

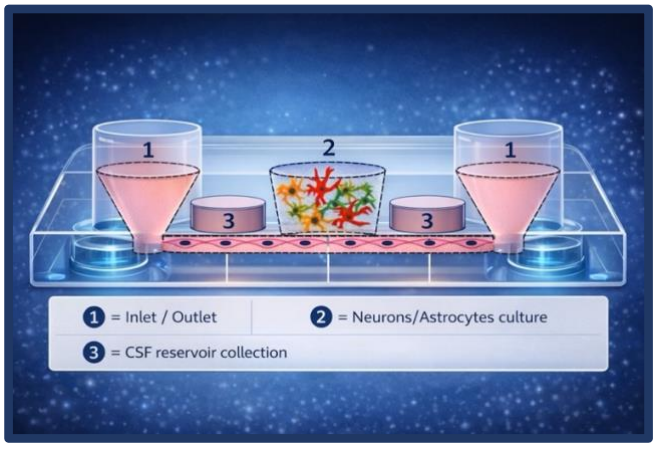

Figure 1. Schematic overview of a microfluidic BBB unit

How is the model generated?

1. GLUT1-BBB CIVM is a mouse stem cell-based (mESCs) organ-on-chip (OoC) model. First step for model development is the derivation of mESCs from GLUT1 knock out heterozygous mice by the use of a standardized procedure.

2. mESCs are then differentiated in brain endothelial cells, neurons and astrocytes. The functionality of brain endothelial cells to form a intact BBB is evaluated by measuring TEER and by permeability assays.

3. Differentiated cells are assembled together onto a BBB microfluidic platform, provided by Finnadvance (Finland), a partner in the PRIM-TECH3R project.

4. The platform contains 24 culture units (24 separate BBB models) and can be implemented up to 128 separate units in a 384 plate. Each unit consists of a microchannel and a culture chamber. Each culture chamber is divided horizontally into two parts by an endothelial barrier membrane that separates the lower part from the upper part of the chamber to allow for a 3D construct. Each culture chamber is located between two reservoirs (inlet-outlet) for the culture medium and two reservoirs for the collection of the medium after passage throughout the endothelial barrier (CSF in the model, to be compared to CSF *in vivo*).

5. To develop the model, endothelial cells are plated through the inlet reservoirs. The plate is inverted and kept shaking to form the endothelial barrier. Then the pre-differentiated neurons and astrocytes are dissociated, mixed in a 5:1 ratio and plated in the upper part of the culture chamber.

Potential applications:

The GLUT1 BBB model might be applied to biomedical investigations regarding GLUT1 DS syndrome but also neurodegenerative diseases were glucose transport through GLUT1 is impaired. Moreover, the model can be adapted to other mouse genetic models for specific diseases that affect the BBB. For both GLUT1 and other disease BBB models several scalable readouts can be generated. Here follows just few of the potential BBB CIVM readouts:

Measure of BBB integrity:

1. TEER measurement, scalable using AKITA lid TEER device (Finnadvance Ltd)

2. 40 kDa FITC–dextran permeability assay, scalable using microplate readers BBB permeability:

3. Fluorescent Glucose permeability assay, scalable using microplate readers

Who provides this model?

The laboratory environment includes fully equipped molecular biology and biochemistry workstations, tissue culture rooms (biosafety level P2), and access to shared spaces. The institute provides advanced core facilities and equipment, including: genomics and molecular biology platforms; Flow cytometry and cell sorting facilities; Confocal and advanced imaging systems; Real-time PCR and sequencing platforms; Tissue culture and 3D model development infrastructure.

IBBC hosts the Italian node of the INFRAFRONTIER Infrastructure, providing collections of mutant models/associated data and platforms to study genome's function in health and disease. INFRAFRONTIER-European Mouse Mutant Archive (EMMA), a world-leading repository, ensures archiving and distribution of mutant models. Whole-organism, analysis of genotype-phenotype interactions is provided by dedicated INFRAFRONTIER mouse clinics, allowing bottom-up access for individual scientists and top-down large-scale initiatives, including the International Mouse Phenotyping Consortium (IMPC).

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References:

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