Development and Characterization of a Dynamic Triculture Blood-Brain Barrier Model

Simona Dobiasova[1], Mohamed Abd Naceur Ammar[1, 2], Iris Chiara Salaroglio[1], Pierre-Olivier Couraud[3], Chiara Riganti[1]

[1] Molecular Biotechnology Center "Guido Tarone", Department of Oncology, University of Torino, Piazza Nizza 44, 10126, Torino, Italy

[2] Research Laboratory LR21ES03, Oncogenesis and Tumor Progression, Medicine Faculty of Sousse, University of Sousse, Sousse, Tunisia

[3] Institute Cochin, 22 Rue Méchain, 75014 Paris, France

contact: simona.dobiasova@unito.it

Introduction

The blood-brain barrier (BBB) limits drug delivery to the central nervous system through tight junctions and efflux transporters. However, conventional *in vitro* BBB models often lack key neurovascular interactions and flow conditions, limiting their ability to reproduce BBB properties *in vivo* [1]. Incorporating astrocytes and pericytes together with flow may improve BBB maturation and functionality.

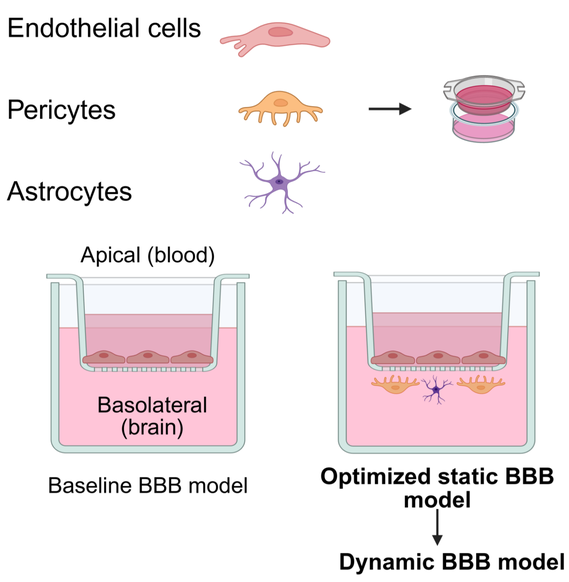
Aim of study

To develop and characterize a dynamic triculture human BBB model combining hCMEC/D3 endothelial cells with primary human astrocytes and pericytes, and to evaluate the contribution of supporting cells and flow conditions to barrier properties.

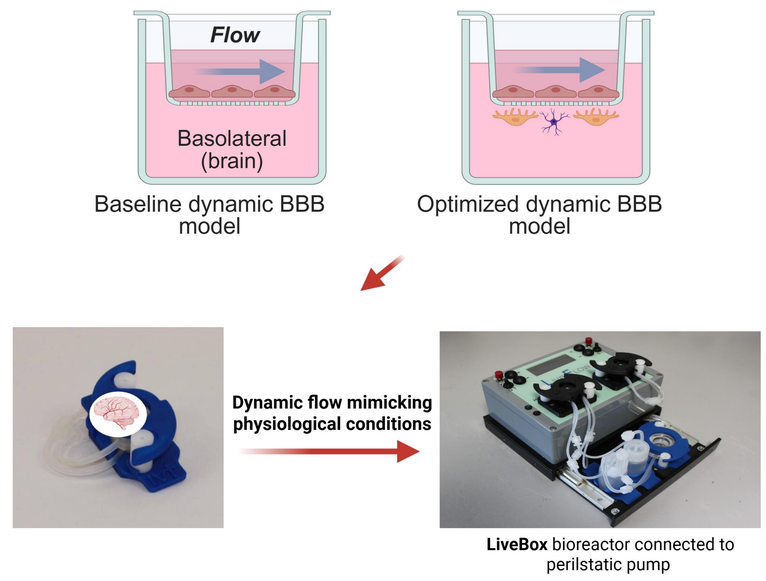
Methodology

Static BBB models were established using hCMEC/D3 endothelial cells cultured alone or in co-culture with primary human astrocytes and pericytes (A/P). The optimized triculture model was subsequently transferred to dynamic culture using the IVTech LiveBox system. Barrier properties under static and dynamic conditions were assessed by permeability to Luciferin Yellow (LY), doxorubicin (DOX) and mitoxantrone (MIT), and by analysis of BBB-associated gene and protein expression.

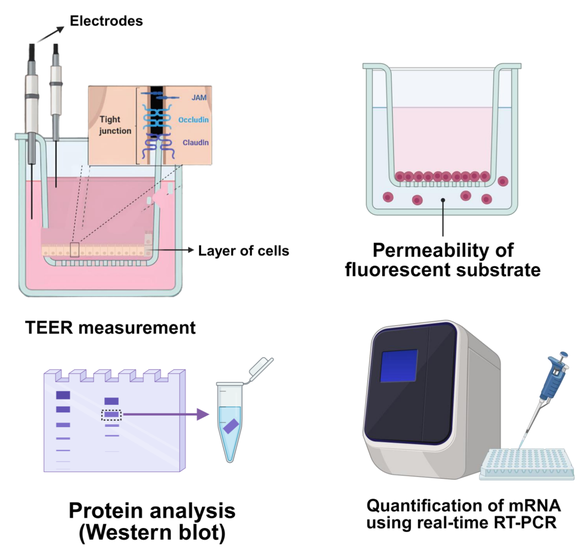
1. BBB static model



2. BBB dynamic model



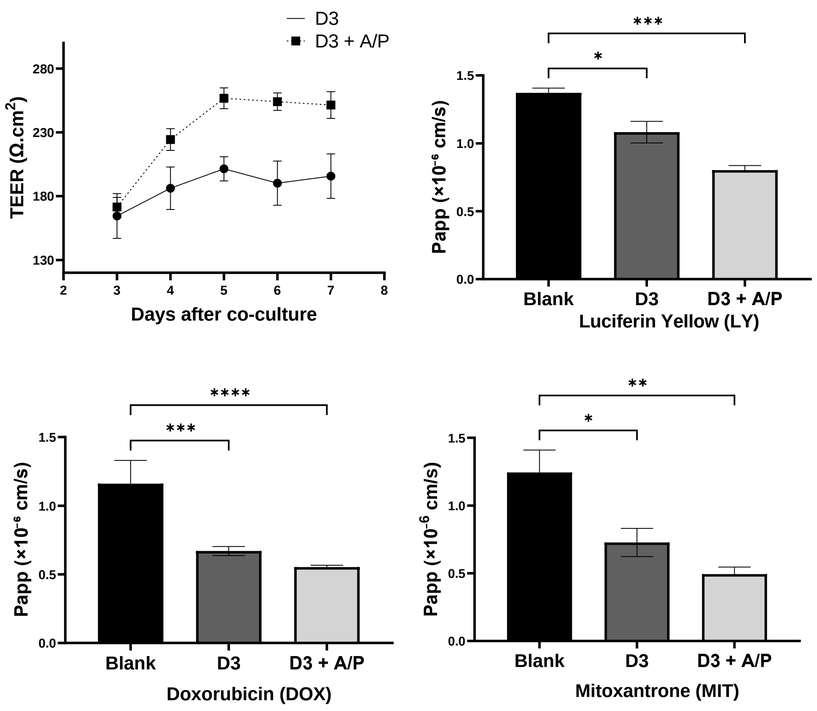
3. Functional and molecular characterization



Results

1. Astrocytes and pericytes enhance BBB properties under static conditions

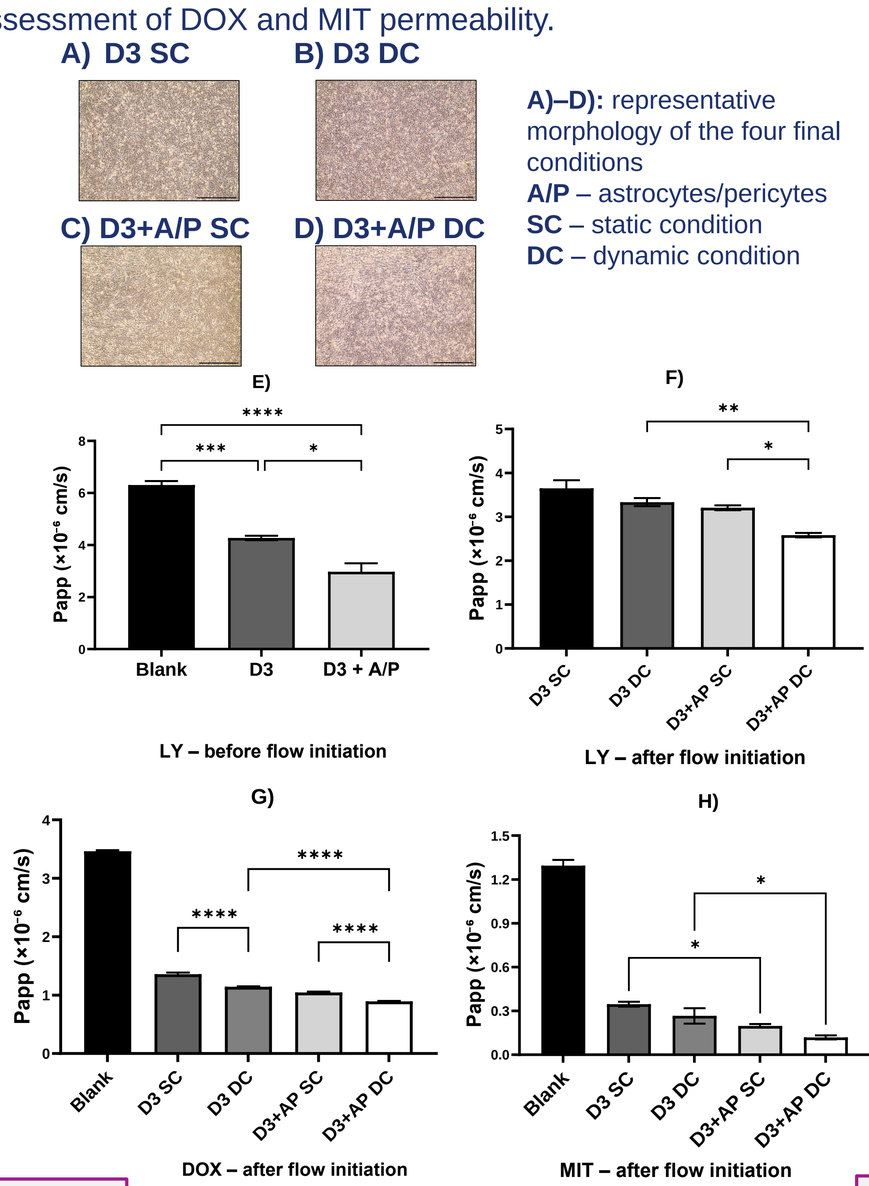
Co-culture with astrocytes and pericytes increased TEER and decreased LY, DOX and MIT permeability, demonstrating improved BBB properties.



Dynamic culture (DC) decreased LY, DOX and MIT permeability compared with static culture (SC), with the lowest permeability consistently observed in the dynamic triculture model.

2. Dynamic culture further improves barrier function

The effect of flow on barrier properties was evaluated in hCMEC/D3 monoculture and triculture models by comparing LY permeability before and after flow initiation, followed by assessment of DOX and MIT permeability.

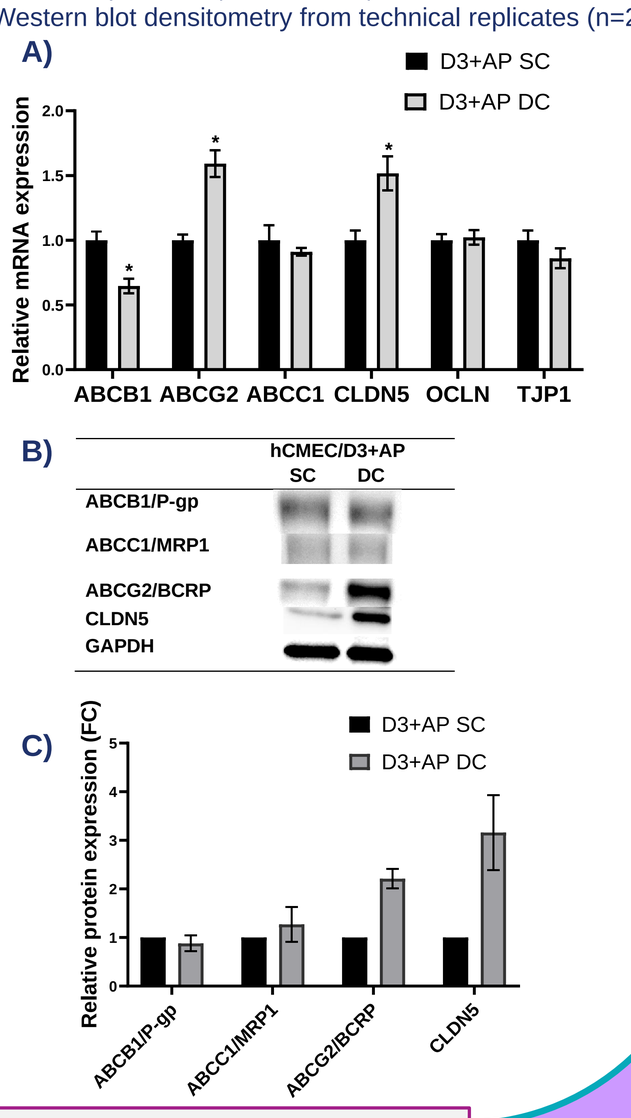


A)–D): representative morphology of the four final conditions
A/P – astrocytes/pericytes
SC – static condition
DC – dynamic condition

3. Dynamic culture increases CLDN5 and ABCG2/BCRP expression

Dynamic culture increased CLDN5 and ABCG2/BCRP expression in the triculture model, whereas changes in other BBB-associated markers were less pronounced.

A) Gene expression (RT-qPCR)
B) Protein expression (Western blot)
C) Western blot densitometry from technical replicates (n=2)



Conclusions

- ✓ **Human astrocytes and pericytes strengthen hCMEC/D3 barrier properties.**
- ✓ **Dynamic models show an increase in barrier function, with the most restrictive phenotype achieved in the triculture model.**
- ✓ **Flow-induced changes in CLDN5 and ABCG2/BCRP expression support the functional improvement in barrier properties.**
- ✓ **The dynamic triculture model provides an improved platform for BBB and drug permeability studies under more physiologically relevant conditions.**

Acknowledgements

This project has received funding from the European Union's Horizon Europe research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 101151942 — GBM4EPI.

Marie Skłodowska-Curie Actions

Funded by the European Union

References: 1) Ammar, Dobiasova *et al*. Modeling blood–brain barrier–glioblastoma interactions: implications for chemoresistance and therapeutic targeting, Fluids and Barriers of the CNS (2026), 23(1):87, https://doi.org/10.1186/s12987-026-00816-3