

Modeling systemic therapeutics delivery: coupling human in vitro BBB models with brain organoids for preclinical screening of RNA delivery

Wang-Xia Wang^{1,2,3,4}, Jahid Islam¹, Paresh Prajapati¹, Guogen Mao¹

¹Sanders-Brown Center on Aging, ²Spinal Cord and Brain Injury Research Center,

³Pathology and Laboratory Medicine, ⁴Neuroscience, College of Medicine, University of Kentucky
Lexington, KY 40536, USA

Monday-0073



Background

- Neurological drug development remains exceptionally challenging, and one of the most significant barriers to effective delivering CNS therapeutics is the blood–brain barrier (BBB).
- Traditional screening approaches, primarily 2-D cell culture and animal models, frequently fail to predict human outcomes due to species differences, disease complexity, and mismatched pharmacokinetics.
- To address these limitations, we are developing a fully human-derived testing platform that integrates an in vitro triculture BBB model with iPSC-derived human brain organoid culture. This system mimics systemic drug delivery, ensuring that any candidate biomolecule/compound is first evaluated for its ability to cross the BBB as well as its therapeutic effects in a human-relevant context.

Methods

- The combined BBB–organoid model was assembled using a Transwell system, with the BBB cultured on the Transwell and the organoids positioned beneath the Transwell (**Fig. 1, left panel**).
- An in vitro BBB model was established using hCMEC/D3 cells seeded on the apical side of 24-well Transwell inserts, with primary astrocytes and pericytes cultured on the basolateral side to promote tight junction formation.
- BBB integrity and permeability were then assessed using ZO-1 tight-junction marker, fluorescently labeled 3-kDa/75-kDa dextran permeability, and Trans-Epithelial Electrical Resistance assays.
- Human brain organoids were generated from iPSCs (**Fig. 1, right panel**).

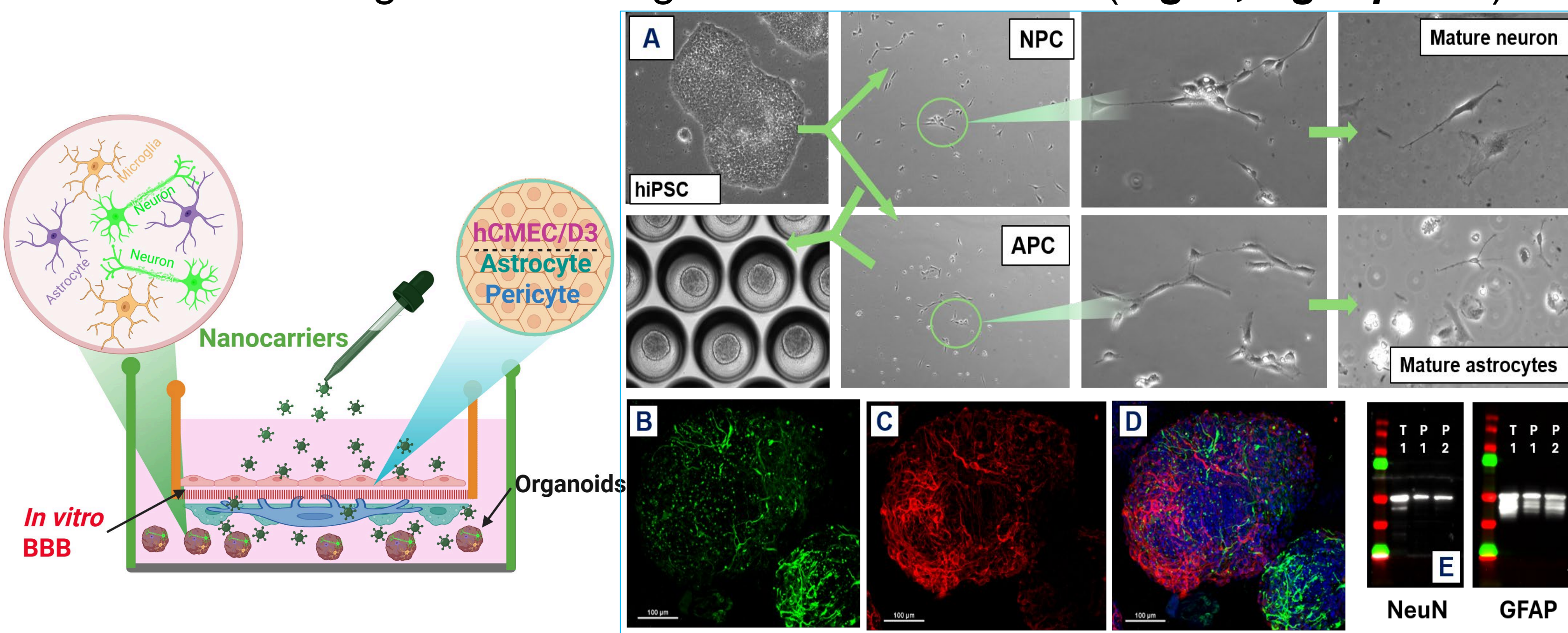


Fig. 1. Left panel: Integrated in vitro BBB-organoid model for testing nanocarrier BBB-penetration. Right panel: General procedure for human iPSC-derived brain organoids. A. iPSCs are first guided into neural induction and differentiated into neural progenitor cells (NPC), further switched using astrocyte differentiation medium to produce astrocyte progenitor cells (APC). NPCs and APCs are then matured to neurons and astrocytes. B–D. IF staining of brain organoids showing astrocytes (green, GFAP) and neurons (red, MAP2); D. merged image with DAPI nuclear staining (blue). E. WB analysis confirming the presence of key markers of brain cell types in the organoids.

Results

- Evaluation of model performance

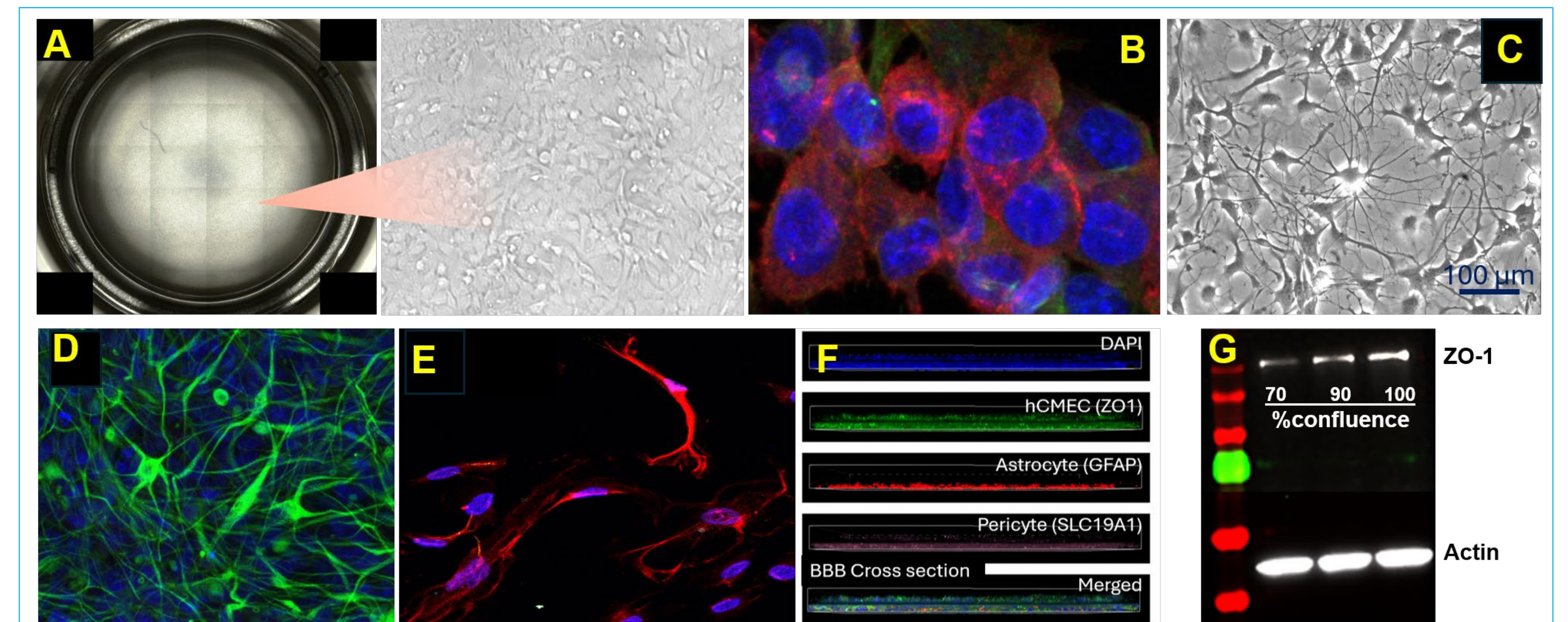


Fig. 2 In vitro BBB model established in 24-well Transwell inserts. hCMEC/D3 endothelial cells seeded on the apical side formed tight junctions, as shown by ZO-1 staining (A,B). Primary human astrocytes (GFAP+, C, D) and pericytes (SLC19A1+, E) were cultured on the basal side. A cross-section of the BBB model is shown in (F). WB analysis showed ZO-1 expression increased with endothelial cell confluency (E), indicating tight junction maturation.

- In vitro BBB permeability assays

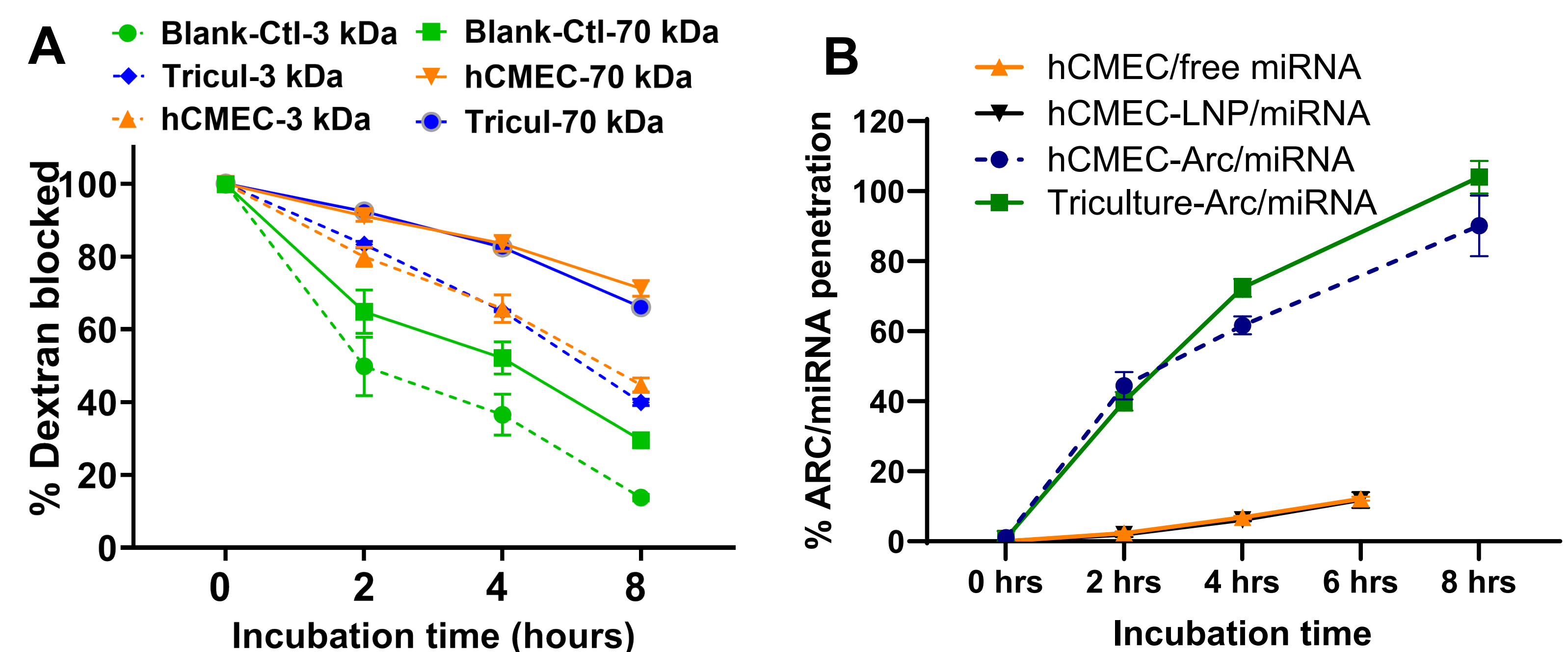


Fig. 3. A. Permeability of the in vitro BBB model. hCMEC/D3 endothelial cells were cultured on the apical side of Transwell inserts either alone (hCMEC) or in triculture with astrocytes and pericytes on the basolateral side (Tricul). After barrier formation, permeability was assessed using 3- and 70-kDa fluorescent dextrans. Both the hCMEC monolayer and triculture model effectively restricted dextran diffusion, demonstrating functional BBB properties. B. Evaluation of nanoparticle transport across the in vitro BBB. RVG-engineered Arc capsid nanocarriers (ARC) and lipid nanoparticles lacking a BBB-targeting ligand (LNP) were loaded with fluorescently labeled miRNA and applied to the apical chamber. Arc nanocarriers efficiently transported the miRNA cargo across the BBB, achieving nearly complete delivery within 8 h, whereas LNPs or free miRNA showed minimal BBB penetration.

Conclusion

This integrated BBB–organoid model provides a robust, human relevant in vitro platform suitable for preclinical CNS drug screening.

Acknowledgements: This work is partially supported by National Institute on Aging (R01AG082142) and SBCoA Startup fund.