26th – 28th
March, 2026

HUN-REN Biological Research Centre Szeged

8th Mini-Symposium on the BBB from Basic to Clinical Research

sponsored by the Hungarian Neuroscience Society

ABSTRACT BOOKLET

Welcome message

8th Mini-Symposium On The Blood-Brain Barrier „From Basic To Clinical Research" From molecular mechanisms to clinical insight — advancing BBB research. The „8th Mini-Symposium on the Blood–Brain Barrier" brings together researchers from across the BBB field to share ideas, data, and perspectives spanning basic, translational and clinical research. The meeting provides a focused and interactive forum for knowledge exchange.

The scientific program covers a broad range of topics, including BBB in aging, glycocalyx, claudin-5 regulation, neurodegenerative, infectious, and other CNS diseases, metastasis, neuroimmune interactions, toxicology, and stem cell research. Emerging and innovative approaches such as lab-on-a-chip technologies, brain organoids, BBB protection strategies, brain targeting and neurovascular unit biology will also be featured.

By bringing together diverse disciplines and methodologies, the symposium aims to provide a niche for discussion, collaboration, and new directions in BBB research.

We warmly welcome you to Szeged and look forward to an inspiring and engaging meeting!

Sincerely,
Mária A. Deli, PhD
Fruzsina R. Walter, PhD

Organized by
Biological Barriers Research Group,
HAS-HUN-REN BRC „Momentum" Translational Lab-on-a-chip
Models Research Group, Institute of Biophysics,
HUN-REN Biological Research Centre Szeged

Sponsored by
Hungarian Neuroscience Society,
PharmaCo-Cell Ltd.,
HUN-REN Biological Research Centre

Scientific Programme Committee & Organizing committee
Mária A. Deli – Institute of Biophysics, HUN-REN BRC
Fruzsina R. Walter – Institute of Biophysics, HUN-REN BRC

Contact
HUN-REN Biological Research Centre Szeged
Address: 6726 Szeged, Temesvári krt. 62., Hungary
E-mail: deli.maria@brc.hu and walter.fruzsina@brc.hu
Conference homepage:https://bbbminisymp-szeged2026.org/

Scientific Programme

Oral Presentations
March 26, 2026

15:00 –	**Registration**

16:00 – 16:10	**Opening remarks** Maria Deli & Fruzsina Walter *(Biological Barriers Research Group and Momentum Translational Lab-on-a-chip Models Research Group; Institute of Biophysics, HUN-REN Biological Research Centre Szeged, Hungary)*
16:10 – 17:10	**Keynote I.** *Chair: Maria Deli,* HUN-REN BRC **Diseases of the blood-brain barrier and their treatments** William A. Banks *(Geriatric Research Educational and Clinical Center, Veterans Affairs Puget Sound Health Care Center, and Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington School of Medicine, USA)*
17:10 – 18:10	**Keynote II.** *Chair: István Krizbai,* HUN-REN BRC **Opportunities for anti-edema treatment explored in experimental acute ischemic stroke** Eszter Farkas *(HCEMM-USZ Cerebral Blood Flow and Metabolism Research Group; Department of Cell Biology and Molecular Medicine, University of Szeged, Hungary)*

18:15 –	**Welcome Dinner** HUN-REN Biological Research Centre, Aula

Oral Presentations

March 27, 2026

8:00 –	**Registration**

9:00 – 11:00	**Session I. – Novel BBB Models** Chair: Andrej Kovac *Institute of Neuroimmunology, SAS, Bratislava, Slovakia*
9:00 – 9:40	**Keynote III.** **Intrinsic blood–brain barrier dysfunction drives progressive multiple sclerosis revealed by hiPSC-derived models** Hideaki Nishihara *(Department of Neurology, Yamaguchi University Graduate School of Medicine)*
9:40 – 10:00	**Endothelial tight junctions and cell-matrix adhesions reciprocally control blood-brain barrier integrity** Gergő Porkoláb *(Biological Barriers Research Group, HUN-REN Biological Research Centre Szeged, HU; Smurfit Institute of Genetics, Trinity College and FutureNeuro Research Ireland Centre, Trinity College Dublin, Ireland)*
10:00 – 10:20	**An isogenic self-assembled blood–brain barrier model coupled to cerebral organoids to investigate transport dysregulation in Alzheimer's disease** Sarah Spitz *(Department of Mechanical Engineering and Biological Engineering, Massachusetts Institute of Technology, MA, USA; Technical University, Vienna, Austria)*
10:20 – 10:40	**Human autologous vascularized immunocompetent brain organoids: a translational platform to study the neuro-vascular unit *in vitro*** Clémence Disdier *(Paris-Saclay University, CEA, INRAE, MTS, SPI, Neurovascular Unit Research & Therapeutic Innovation Laboratory, Gif-sur-Yvette cedex, France)*
10:40 – 11:00	**Stem cell-derived BBB and brain-on-a-chip platforms to study neurovascular adverse effects and drug penetration** Fruzsina Walter *(Biological Barriers Research Group and Momentum Translational Lab-on-a-chip Models Research Group; Institute of Biophysics, HUN-REN Biological Research Centre Szeged, Hungary)*

11:00 – 11:40	**Coffee Break**

11:40 – 13:00	**Session II. – BBB in Diseases** *Chair: Yoichi Morofuji* *Department of Neurosurgery, Showa Medical University School of Medicine, Tokyo, Japan*
11:40 – 12:00	**Sympathetic denervation attenuates pulmonary edema following experimental aneurysmal subarachnoid hemorrhage by protecting the pulmonary vascular endothelial glycocalyx** Nozomi Sasaki *(Department of Neurosurgery, Gifu University Graduate School of Medicine and Molecular Pharmacology, Department of Biofunctional Evaluation, Gifu Pharmaceutical University, Gifu, Japan)*
12:00 – 12:20	**Central nervous system fibroblasts remodel the cerebrovascular basement membranes through osteopontin signaling in Alzheimer's disease** Akihiko Urayama *(Department of Neurology, McGovern Medical School, University of Texas Health Science Center, Houston, Texas, USA)*
12:20 – 12:40	**CLDN5-related neurological disease** Yosuke Hashimoto *(Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan and Smurfit Institute of Genetics, Trinity College Dublin, Ireland)*
12:40 – 13:00	**Blood–brain barrier dysfunction in tick-borne encephalitis: NS1 as a potential antiviral and therapeutic target** Martin Palus *(Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic; Department of Virology, Veterinary Research Institute; and Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic)*

13:00 – 14:00 Lunch – BRC Cantine

14:00 – 15:30 Poster Session – BRC Aula

15:30 – 16:00 Coffee Break

16:00 – 18:00	**Session III. – Neuroimmune interactions and transport at the brain barriers** *Chair: Szilvia Veszelka* Biological Barriers Research Group, HUN-REN BRC, Szeged, Hungary
16:00 – 16:40	**Keynote IV.** **Microglia modulate neurovascular responses under systemic inflammatory conditions** Ádám Dénes *(Laboratory of Neuroimmunology, HUN-REN KOKI, Budapest, Hungary)*
16:40 – 17:00	**Brain shuttle target expression levels vary by individual, not by brain region, disease, age, or gender** Ana Raquel Santa-Maria (online presentation) *(Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA, USA)*
17:00 – 17:20	**A proof of concept: a delivery system to transport antimicrobial peptides across BBB against neuropathogens** Mangesh Bhide *(Laboratory of Biomedical Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice, Slovakia and Institute of Neuroimmunology, Slovak Academy of Sciences v. v. i., Bratislava, Slovakia)*
17:20 – 17:40	**Expression of alpha smooth muscle actin decreases with ageing and increases upon lumen obstruction in mouse brain pericytes** Fanni Győri *(Neurovascular Unit Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary)*
17:40 – 18:00	**Brain diffusion of Tau and its transport across the blood–CSF barrier in neurodegeneration** Petra Majerova *(Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia)*
18:00 – 18:20	**The histone deacetylase inhibitor suberoylanilide hydroxamic acid promotes blood-brain barrier protection during reperfusion in a cell culture model of ischemic stroke** Zuhao Cui *(Biological Barriers Research Group; Institute of Biophysics, HUN-REN Biological Research Centre Szeged, Hungary)*

18:20 – 18:30	**Closing remarks**

19:00 –	**Dinner (optional)**

March 28, 2026	**10:00 – 21:00**	**Social Program (optional)**

Poster Presentations

March 26, 2026

14:00 – 15:30

Poster 1

Bidirectional communication between brain metastatic cells and cells of the neurovascular unit

Rabiya Bano[1], Csilla Fazakas[1], Adél Lüvi[1], Maryam Naeem[1], Kinga Molnár[1], Attila E. Farkas[1], István Krizbai[1], Imola Wilhelm[1]

[1]HUN-REN Biological Research Centre, Szeged, Hungary

Poster 2

Tick-borne encephalitis virus and its NS1 protein disrupt endothelial monolayer integrity and contribute to endothelial glycocalyx disruption

Monika Čížková[1,2], Marika Davídková[1], Markéta Dvořáková[1], Hana Sehadová[2,3], Veronika Prančlová[1,2], Eliška Kotounová[1,2], Martin Palus[1,4,5]

[1]Biology Centre CAS, Laboratory of Arbovirology, Ceske Budejovice, Czech Republic
[2] University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Republic

Poster 3

Investigation of the effects of antidepressant compounds from traditional chinese medicine on brain endothelial cells, and their permeability across the blood-brain barrier: a China-Hungary collaborative study

Zuhao Cui[1,2], Ana Martins[1], Aniko Szecsko[1,2], Tivadar Kiss[3], Andrea Vasas[3], Fruzsina R. Walter[1], Szilvia Veszelka[1], Attila Hunyadi[3], Gang Chen[4], Maria A. Deli[1]

[1]Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary
[2]Doctoral School of Biology, University of Szeged, Szeged, Hungary

Poster 4

Capillary pericytes regulate vascular tone and local blood flow in inflammation

Tamás Dudás[1,2], Ádám Mészáros[1], Kinga Mészáros-Molnár[1], Attila E. Farkas[1], Imola Wilhelm[1], István Krizbai[1]

[1]Institute of Biophysics, Biological Research Centre, Szeged
[2]Doctoral School of Experimental and Preventive Medicine, University of Szeged, Szeged, Hungary

Poster 5

Extracellular vesicles from triple negative breast cancer cells disrupt the blood–brain barrier via miR-146a-5p- and TGF-β1-mediated downregulation of endothelial Paqr5

Csilla Fazakas[1], Attila G. Végh[1], Tamás Dudás[1,2], Dorina Varga[1], Adél Lüvi[1], Mónika Krecsmarik[1], András Dér[1], Attila E. Farkas[1], István A. Krizbai[1,3,4,*], Imola Wilhelm[1,4,*,#]

[1]Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary
**Authors contributed equally*

Poster 6	**An integrated microphysiological platform for barrier modeling and whole-organoid imaging** Luíza Santa Brígida de Barros Góes[1,2,3], László Dér[1], Emese Bélai[4], Pirity Melinda Katalin[4], Sándor Valkai[1], András Dér[1], Mária A. Deli[1], Fruzsina R. Walter[1,*], Emanuel Carrilho[2,3,*] *[1] Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary* *[2] Instituto de Química de São Carlos, Universidade de São Paulo 400, Brazil* *[3] Instituto Nacional de Ciência e Tecnologia de Bioanalítica Lauro Kubota – INCTBio-LK Campinas SP, Brazil* **Authors contributed equally*
Poster 7	**Microfluidic synthesis and physicochemical characterization of petox-pcl nanoparticles** **S. Göksever**[1,2], B. Küçüktürkmen[1], UC. Oz[1], A. Hunyadi[3,4], A. Bozkır[1] *[1]Ankara University, Department of Pharmaceutical Technology, Ankara, Turkey* *[2]Ankara University, Institute of Health Sciences, Ankara, Turkey* **Authors contributed equally*
Poster 8	**Investigation of a pentapeptide carrier on a culture model of the blood-brain barrier** Ilona Gróf[1], Alexandra Bocsik[1], Milán Szántó[1], Enikő Szabó[2], Imre Norbert[3], Tamás Martinek[3] and Mária A. Deli[1] *[1]Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary*
Poster 9	**A versatile brain-on-a-chip system to study pathological conditions of the central nervous system** Anna E. Kocsis[1,2], Judit P. Vigh[1,2], Ana R. Santa-Maria[1,3], Nóra Kucsápszky[1,2], Silvia Bolognin[4], Jens C. Schwamborn[4], András Kincses[1], Anikó Szecskó[1,2], Szilvia Veszelka[1], Mária Mészáros[1], Emese Bélai[1], Melinda Pirity[1], András Dér[1], Fruzsina R. Walter[1,*], Mária A. Deli[1,*] *[1]HUN-REN Biological Research Centre, Szeged, Hungary* *[2]Doctoral School of Biology, University of Szeged, Szeged, Hungary* **Authors contributed equally*
Poster 10	**Translational research aimed at improving heatstroke diagnosis** Andrej Kovac[1,*], Kazuyuki Miyamoto[2], Dorothy Wasike[1], Petra Majerova[1] *[1]Institute of Neuroimmunology, SAS, Bratislava, Slovakia* *[2]Showa University, Department of Emergency and Disaster Medicine, Tokio, Japan*
Poster 11	**Blood-brain barrier disruption in acute pancreatitis: a clinical and cell-culture study** Nóra Kucsápszky[1,2,3*], Ármin Szeles[1,2*], Ana R. Santa-Maria[1,4], Judit P. Vigh[1,2,3], Luíza Santa Brígida de Barros Góes[1,2,5,6], Anna E. Kocsis[1,2,3], Zoltán Rakonczay[7], Péter Hegyi[8], Mária A. Deli[1], Fruzsina R. Walter[2] *[1]Biological Barriers Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary* *[2]HAS-HUN-REN BRC Lendület "Momentum" Translational Lab-on-a-chip models Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary* *[3]Doctoral School of Biology, University of Szeged, Szeged, Hungary* **Authors contributed equally*

Poster 12

The effects of paracellular barrier tightening on the structure and functions of focal adhesions in a human blood-brain barrier model

Lucien Lemaitre[1*], Ilona Gróf[1*], Gergő Porkoláb[1,2*], Imola Rajmón[1,3], Róbert Horváth[1,3], Mária A. Deli[1]

[1]Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary
[2]Smurfit Institute of Genetics, Trinity College Dublin, D02 VF25 Dublin, Ireland
*Authors contributed equally

Poster 13

Astrocyte-activated NLRP3 inflammasome promotes brain metastasis formation

Adél Lüvi[1], Ádám Mészáros[1], Kinga Molnár[1], Csilla Fazakas[1], Tamás Dudás[1], Attila E. Farkas[1], István A. Krizbai[1], Imola Wilhelm[1]

[1] Biological Research Centre, Szeged, Hungary

Poster 14

Microplastics and the Blood–Brain Barrier

Petra Majerova[1], Andrej Kovac[1]

[1]Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia

Poster 15

Clazosentan suppresses endothelin-1-induced ROS production and metabolic alterations in cerebral capillary pericytes

Hiroki Nagatsuka[1], Genki Chikamatsu[3], Eri Shiozaki[2,3], Yoichi Morofuji[1]

[1]Department of Neurosurgery, Showa Medical University, Tokyo, Japan

Poster 16

Modulation and measurement of endothelial and epithelial barrier integrity by FluidFM

Imola Rajmon[1,2,3], Inna Székács[1], Mária A. Deli[2], Róbert Horváth[1,2]

[1]Nanobiosensorics Laboratory, Centre for Energy Research, Institute of Technical Physics and Materials Science, HUN-REN, 1121 Budapest, Hungary
[2]Institute of Biophysics, Biological Research Centre, Hungarian Research Network, Szeged H-6726, Hungary
[3]Doctoral School of Biology, University of Szeged, Szeged H-6720, Hungary

Poster17

Protection of the blood-brain barrier in the cell culture model of ischemic stroke

Koppány Párdi[1], Anikó Szecskó[1,2], Zuhao Cui[1,2], Gergő Porkoláb[1,+], Zsófia Hoyk[1], Csilla Kovács[1], Janet Folasade Adegbite[1,++], Nárcisz M. Cser[1], László Dér[3], Krisztina Nagy[3,4], Virág Fodor[1], Mária A. Deli[1], Szilvia Veszelka[1]

[1]Biological Barriers Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary

Poster 18

Neuroprotective roles of HSPB1 in a mouse model of Alzheimer's disease

Bettina Rákóczi[1], Zsófia Ruppert[1], Mária Péter[1], Gábor Balogh[1], Ede Migh[1], László Vígh[1], Zsolt Török[1], Melinda E. Tóth[1]

[1]Institute of Biochemistry, HUN-REN Biological Research Centre, Szeged

Poster 19

Traumatic brain injury induces senescence in the cells of the neurovascular unit

Tejal Shreeya[1,2], Zsófia Hernádi[1], Imola Wilhelm[1], Endre Czeiter[3], Krisztina Amrein[3], Zsolt Kristóf Bali[4], Nóra Bruszt[4], István Hernádi[4], Attila E. Farkas[1], István Krizbai[1]

[1]Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungarian Research Network, Szeged, Hungary
[2]Doctoral School of Experimental and Preventive Medicine, University of Szeged, Szeged, Hungary

Poster 20

Complex temporal activity patterns replayed with fast 3D acousto-optical stimulation for partial visual restoration

Gergely Szalay[1,2*], Linda Judák[1,2*], Pál Maák[3], András Fehér[1], Andrius Plauska[1], Abhrajyoti Chakrabarti[1], Gábor Juhász[1], Balázs Tarján[1], Máté Veress[1], Zoltán Szadai[1,2], Balázs Rózsa[1,2,4]

[1]BrainVisionCenter, Budapest-1094, Hungary
[2]Laboratory of 3D functional network and dendritic imaging, HUN-REN, Budapest-1083, Hungary
**Authors contributed equally*

Poster 21

The protection of the blood-brain barrier by a small-molecule cocktail, cARLA in a cell culture model of ischemic stroke

Anikó Szecskó[1,2], Koppány Párdi[1], Zuhao Cui[1,2], Gergő Porkoláb[1,3], Zsófia Hoyk[1], Csilla Kovács[1], Nárcisz M. Cser[1], Krisztina Tóth[4], Ádám Dénes[4], Csilla Sajben[5], Roland Tengölics[5], Mária A. Deli[1], Szilvia Veszelka[1]

[1]BrainVisionCenter, Budapest-1094, Hungary
[2]Laboratory of 3D functional network and dendritic imaging, HUN-REN, Budapest-1083, Hungary
**Authors contributed equally*

Poster 22

Increased BBB permeability to a-synuclein in dextran sulfate sodium-induced colitis mice

Fuyuko Takata[1], Junko Mizoguchi[1], Takuro Iwao[1], Yasuyoshi Tanaka[1], Akio Nakashima[2], Kazunori Sano[3], Osamu Imakyure[2], Shinya Dohgu[1]

[1]Department of Pharmaceutical Care & Health Sciences, Faculty of Pharmaceutical Sciences, Fukuoka University
[2]Department of Pharmacy, Fukuoka University Chikushi Hospital
[3]Department of Physiology and Pharmacology, Faculty of Pharmaceutical Sciences, Fukuoka University

Poster 23

Albumin-induced blood-brain barrier dysfunction and its role in neuroinflammation relevant to epileptogenesis

Daiki Uchida[1], Yoichi Morofuji[2], Daisuke Watanabe[3], Shiro Baba[1], Takayuki Matsuo[1]

[1]Department of Neurosurgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Poster 24

Interfacial water organization in glycocalyx model systems: towards understanding blood-brain barrier hydration structure

Dános Sebestyén Varga[1,2,3,4], Ilona Gróf[1], Lóránd Kelemen[1], Mária Anna Deli[1], Róbert Horváth[1,3], Zsuzsanna Heiner[4], András Dér[1]

[1]Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary
[2]Doctoral School of Multidisciplinary Medical Sciences, University of Szeged, Szeged, Hungary
[3]Nanobiosensorics Laboratory, HUN-REN Centre for Energy Research, Budapest, Hungary
[4]Institute of Chemistry and School of Analytical Sciences Adlershof, Humboldt-Universität zu Berlin, Berlin, Germany

Poster 25

Effects of clinically used iodinated contrast agents on blood-brain barrier integrity after oxygen-glucose deprivation

Judit P. Vigh[1,2,*], Anna E. Kocsis[1,2,*], Ilona Gróf[1,*], Ana Raquel Santa-Maria[1,3], Yuki Matsunaga[4], Daisuke Watanabe[5], Yoichi Morofuji[6], Mária A. Deli[1,*], Fruzsina R. Walter[1,*]

[1]Biological Barriers Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary
[2]Doctoral School of Biology, University of Szeged, Szeged, Hungary
**Authors contributed equally*

Poster 26

Development of a human *in vitro* blood-brain barrier model for pharmacoresistant epilepsy

Emílie Kučerová[1], Jitka Viktorová[1]

[1]University of Chemistry and Technology Prague, Technická 5, 166 28, Prague 6, Czechia

Poster 27

Protective effects of isonicotinamides on BBB integrity

Katja Vuković[1], Valentina Bušić[2], Dajana Gašo Sokač[2], Maja Katalinić[1], Antonio Zandona[1]

[1]Division of Toxicology, Institute for Medical Research and Occupational Health, Zagreb, Croatia

Poster 28

Species- and model-dependent efficacy of barrier-protective compounds under ROS-induced stress

Antonio Zandona[1], Anikó Szecskó[2], Valentina Bušić[3], Dajana Gašo Sokač[3], Maja Katalinić[1], Mária Deli[2], Szilvia Veszelka[2]

[1]Division of Toxicology, Institute for Medical Research and Occupational Health, Zagreb, Croatia

Poster 29

Integrated proteomic analysis of resected tumor tissues and longitudinally collected plasma from patients with glioblastoma

Xiuyuan Zhan[1], Adam McGlinchey[2], Angela Garcia-Gallardo[2], Jeffrey O'Callaghan[2], Yosuke Hashimoto[1], Yasuo Uchida[1], Kieron Sweeney[3], Donncha O'Brien[3], Matthew Campbell[2]

[1]Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan

PLENARY SESSION ABSTRACTS

Diseases of the Blood-brain Barrier and their treatments

William A. Banks

Geriatric Research Educational and Clinical Center, Veterans Affairs Puget Sound Health Care Center, and Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington School of Medicine

The blood-brain barrier (BBB) protects and nourishes the central nervous system (CNS) while also regulating the homeostatic environment of the CNS. The BBB does so through many mechanisms that result in limited permeability to and selective transport of blood-borne substances, secretion of substances, enzymatic activity, diapedesis, contributions to the glycocalyx and basement membranes, and other activities. This complexity results in many dysfunctions and diseases that center on the BBB. As this list of BBB dysfunctions grows, so does an understanding of how to therapeutically intervene at the level of the BBB. Perhaps the oldest example of a BBB mediated condition is that of fever. Lipopolysaccharide (LPS) or interleukin-1 acting at the luminal surface of brain endothelial cells (BECs) induces release of prostaglandins from the brain side of the BBB, inducing fever. Acetylsalicylic acid or nonsteroidal anti-inflammatory drugs (NSAIDs) block fever by inhibiting BEC cyclooxygenases. Inflammation induced by LPS or cytokines can also disrupt the BBB and NSAIDs and the anti-oxidant N-acetylcysteine can protect the BBB from inflammation-induced disruption. Depression arising from inflammation is mediated by the increased blood-to-brain transport of kynurenine by the BBB's large neutral amino acid transport system. Competitive blockade of kynurenine transport by the large neutral amino acid leucine prevents inflammation-induced depression. Traumatic brain injury results in a series of openings and closings of the BBB which are related to neuroinflammation. Treatment with the nitric oxide inhibitor L-NAME protects the BBB from disruption. Insulin transport is modulated by inflammation via nitric oxide dependent pathways. Whether insulin transport is increased or decreased by inflammation depends on which of the stimulated nitric oxide pathways predominates. Insulin in the brain is mainly peripheral insulin which has been transported across the BBB. CNS insulin has modest metabolic effects and much more robust effects on cognition and neuroprotection. As such, nitric oxide inhibitors and simulators of BBB insulin transport could have effects on cognition and on metabolism. These are a few examples of the way in which therapeutics regulate BBB function and dysfunction in health and disease and provide avenues for treatment of disease states that involve the BBB.

Opportunities for anti-edema treatment explored in experimental acute ischemic stroke

Eszter Farkas[1,2], Réka Tóth[1,2], Anna Törteli[1,2], Melinda Szabó[2], Noémi Kovács[3,4], Ildikó Horváth[4], Rita Frank[1,2], Domokos Máthé[3,4], Ákos Menyhárt[1,2]

[1]HCEMM-USZ Cerebral Blood Flow and Metabolism Research Group, HCEMM Nonprofit Ltd., Szeged, Hungary
[2]Department of Cell Biology and Molecular Medicine, University of Szeged, Szeged, Hungary
[3]HCEMM-SU In Vivo Imaging Advanced Core Facility, Budapest, Hungary
[4]Department of Biophysics and Radiation Biology - HUN-REN TKI, Semmelweis University, Budapest, Hungary

Cerebral edema and neurovascular dysfunction are reliable predictors of outcome after acute ischemic stroke (AIS), yet effective targeted therapies remain limited. We investigated the therapeutic potential of trifluoperazine (TFP), an FDA-approved antipsychotic and calmodulin inhibitor that modulates astrocytic aquaporin-4 expression. TFP administered after recanalization in a mouse model of AIS reduced infarct volume and improved early neurological recovery. Importantly, TFP restored neurovascular coupling and enhanced cerebral blood flow responses to spreading depolarizations, indicating improved cerebrovascular function. Mechanistic studies in acute brain slice preparations demonstrated that TFP attenuates cytotoxic tissue swelling, suppresses spreading depolarizations, reduces aquaporin-4 expression and preserves neuronal integrity under osmotic stress. These findings suggest that transient modulation of astrocytic cytotoxic edema and vascular reactivity represents a viable strategy to improve early stroke outcomes. Given its established clinical use, TFP emerges as a promising candidate for therapeutic repurposing in AIS.

Intrinsic blood–brain barrier dysfunction drives progressive multiple sclerosis revealed by hiPSC-derived models

Hideaki Nishihara[1], Seiji Okada[1], Jun Nagamatsu[1], Ayano Sasakura[1], Junyoung Kang[1], Kinya Matsuo[1,2]

[1]*Department of Neurology, Yamaguchi University Graduate School of Medicine*
[2]*Yamaguchi Prefectural Grand Medical Center*

Background:

Multiple sclerosis (MS) is characterized by inflammatory relapses and progressive neurodegeneration. While disease-modifying therapies are effective for relapsing forms, treatment options for progressive MS remain limited. We previously established an in vitro blood–brain barrier (BBB) model using hiPSC-derived brain microvascular endothelial-like cells generated by the extended endothelial cell culture method (EECM-BMECs) and demonstrated that intrinsic BBB dysfunction can act as an independent determinant of MS disease subtype.

Methods:

We expanded our hiPSC-derived EECM-BMEC modeling to include 17 MS patients representing various subtypes (benign MS, RRMS, SPMS, and PPMS) and 4 healthy controls. BBB integrity was assessed by evaluating: (1) junctional localization of claudin-5, (2) permeability to sodium fluorescein (NaFl), and (3) cell surface expression of the adhesion molecules ICAM-1 and VCAM-1. RNA sequencing was performed to identify candidate molecules involved in BBB disruption, followed by functional validation.

Results:

NaFl permeability was elevated in SPMS- and PPMS-derived BBB models and showed variability among RRMS-derived EECM-BMECs. Disruption of claudin-5 junctional localization correlated with increased BBB permeability across all MS subtypes. ICAM-1 and VCAM-1 expression was upregulated in EECM-BMECs derived from all MS patients. RNAseq analysis identified PHGDH as a candidate molecule associated with BBB disruption in MS. PHGDH expression was reduced in MS-derived EECM-BMECs, and PHGDH knockdown in healthy control–derived cells reproduced MS-like BBB dysfunction, including decreased impedance and claudin-5 disruption. As direct restoration of PHGDH expression in MS-derived cells proved difficult, we identified serpinE1 as an upregulated downstream molecule. Knockdown of serpinE1 in MS-derived EECM-BMECs led to sustained impedance and recovery of claudin-5 expression.

Conclusion:

These findings support the concept that intrinsic BBB dysfunction is a key contributor to progressive MS and identify PHGDH-related pathways, including serpinE1, as potential therapeutic targets for restoring BBB integrity.

Endothelial tight junctions and cell-matrix adhesions reciprocally control blood-brain barrier integrity

Gergő Porkoláb[1,2,3], Lucien Lemaitre[1], Beatrix Magyaródi[4], Imola Rajmon[1,4,5], Tibor Novák[6], Bálint H. Kovács[6], Ilona Gróf[1], Anikó Szecskó[1,5], Kinga Dóra Kovács[4], Enikő Farkas[4], Boglárka Kovács[4], Inna Székács[4], Attila G. Végh[1], Yosuke Hashimoto[2], Chris Greene[2,7,8], Adam McGlinchey[2], Maxime Culot[9], David C. Henshall[7,8], Kieron J. Sweeney[10], Donncha F. O'Brien[10], Szilvia Veszelka[1], Miklós Erdélyi[6], Matthew Campbell[2,3,*], Róbert Horváth[1,4,*], Mária A. Deli[1,*]

[1]*Institute of Biophysics, Biological Research Centre, Hungarian Research Network, H-6726 Szeged, Hungary*
[2]*Smurfit Institute of Genetics, Trinity College Dublin, D02 VF25 Dublin, Ireland*
[3]*FutureNeuro Research Ireland Centre, Smurfit Institute of Genetics, School of Genetics and Microbiology, Trinity College Dublin, D02 VF25 Dublin, Ireland*
[4]*Nanobiosensorics Laboratory, Institute of Technical Physics and Materials Science, Centre for Energy Research, Hungarian Research Network, H-1121 Budapest, Hungary*
[5]*Doctoral School of Biology, University of Szeged, H-6720 Szeged, Hungary*
[6]*Department of Optics and Quantum Electronics, University of Szeged, H-6720 Szeged, Hungary*
[7]*Department of Physiology & Medical Physics, RCSI University of Medicine and Health Sciences, D02 YN77 Dublin, Ireland*
[8]*FutureNeuro Research Ireland Centre, Department of Physiology & Medical Physics, RCSI University of Medicine and Health Sciences, D02 YN77 Dublin, Ireland*
[9]*Laboratoire de la Barriére Hémato-Encéphalique, Université d'Artois, 62307 Lens, France*
[10]*Department of Neurosurgery, Beaumont Hospital, D09 V2N0 Dublin, Ireland*

Brain endothelial cells (ECs) rely on mechanical cues to provide a physical barrier that protects the brain. Yet how ECs integrate forces to establish and maintain the blood-brain barrier (BBB) remains poorly understood. Here, we show that the two main endothelial force-bearing systems, tight junctions and cell-matrix adhesions, reciprocally control BBB integrity. Using a combination of super-resolution imaging and biophysical techniques, we reveal increasing mechanical loads on cell-cell junctions vs. cell-matrix adhesions in human stem cell-derived ECs during BBB maturation. This force redistribution is enabled by cytoskeletal remodeling, a compacted pattern of the tight junction protein claudin-5, and the emergence of specialised perinuclear cell-matrix adhesions. Mechanistically, we find an inverse relationship between claudin-5 levels and the expression of key cell-matrix adhesion proteins zyxin and vinculin *in vitro* and in mice. Finally, we demonstrate that this mechanobiological signature associated with BBB maturation is reversed upon BBB dysfunction after seizures in mice and in human patients with temporal lobe epilepsy. Collectively, our findings establish a novel interplay between mechanoresponsive elements in brain ECs, with implications for BBB stabilisation therapy in epilepsy.

An isogenic self-assembled blood–brain barrier model coupled to cerebral organoids to investigate transport dysregulation in Alzheimer's disease

Sarah Spitz[1], Francesca Pramotton[1], Maisam Mitalipova[2], Zhengyu Zhang[1], Honghao Cao[2], Zhengpeng Wan[1], Sixian You[2], Rudolf Jaenisch[2], Roger Kamm[1]

[1]*Department of Mechanical Engineering and Biological Engineering, Massachusetts Institute of Technology*
[2]*Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology*
[3]*The Whitehead Institute, 9 Cambridge Center, Cambridge, MA 02142, USA*

The human blood-brain barrier (BBB) obstructs approximately 98% of small-molecule drugs and nearly all biologics from entering the brain, rendering it the primary obstacle to effective therapeutic intervention in the central nervous system. [1] Thus, advancing therapeutic strategies relies on biomimetic models that accurately replicate the barrier's transport mechanisms across both physiological and pathological states. Self-assembled vascular networks have emerged as a leading model for studying the BBB, leveraging natural vascular development processes to create systems that replicate the native architecture and functional characteristics of cerebral capillaries in vitro. However, current microvascular models typically rely on non-isogenic and mixed-cell sources, compromising reproducibility and limiting their applicability in studying neurovascular pathologies on a patient-specific level.[2]

To address these limitations, we developed a fully perfusable, isogenic, self-assembled vascular BBB network model by systematically co-culturing human induced pluripotent stem cell (iPSC)-derived astrocytes, neural crest-derived pericytes, and endothelial cells within a compartmentalized, polydimethylsiloxane-based microfluidic device. This model replicates physiological capillary morphologies and achieves permeability values closely aligned with in vivo measurements (2×10^{-7} cm/s for 10 kDa dextran). Beyond size-dependent variations in bidirectional barrier permeability (3–150 kDa dextrans), the model demonstrates active efflux function, and substrate-specific transport behavior (e.g., MEM-189, 13E4), validated by both ELISA and fluorescence imaging. Receptor-mediated transcytosis dynamics are further characterized using a simultaneous, label-free, autofluorescence multi-photon imaging approach.

To explore pathological interactions within the neurovascular unit, the microvascular BBB model is interconnected to microglia-embedded human cerebral organoids harboring a single familial Alzheimer's disease-associated mutation. These neurovascular co-cultures exhibited impaired barrier integrities and dysregulated transport of amyloid β-42 peptides, a hallmark of Alzheimer's disease.

In conclusion, this isogenic, self-assembled vascular BBB model provides a robust and physiologically relevant system to study transport dynamics and pathological disruptions, providing an essential resource for developing targeted treatments for disorders of the central nervous system.

Sources:
[1] Pardridge WM. (2005). NeuroRx. 2(1):3-14. doi: 10.1602/neurorx.2.1.3.
[2] F. M. Pramotton, S. Spitz, R. D. Kamm. (2024). Adv. Sci. 11, 2403892. doi.org:10.1002/advs.202403892

Human autologous vascularized immunocompetent brain organoids: a translational platform to study the neuro-vascular unit *in vitro*

Clémence Disdier[1], Eva Veiss [1], Nathan Metivet[1], Aloïse Mabondzo[1]

[1]*Paris-Saclay University, CEA, INRAE, MTS, SPI, Neurovascular Unit Research & Therapeutic Innovation Laboratory, Gif-sur-Yvette cedex 91191, France*

The blood–brain barrier (BBB) is a highly specialized interface that preserves central nervous system (CNS) homeostasis by tightly regulating molecular exchange between the circulation and neural tissue. Its function relies on coordinated interactions within the neurovascular unit (NVU), and dysfunction of this system is increasingly recognized as a critical contributor to numerous CNS pathologies. Despite its importance, the BBB remains inadequately modeled in human-relevant experimental systems. Human brain organoids (BOs) derived from induced pluripotent stem cells (iPSCs) have emerged as powerful tools to study brain development and disease; however, their limited cellular diversity, particularly the absence of vascularization and microglial populations restricts their applicability for BBB and NVU research.

We first developed vascularized brain organoids (vBOs) generated from a single human iPSC lineage. iPSCs were differentiated into endothelial cells (ECs), which were subsequently incorporated (10%) at the earliest stage of cerebral organoid formation. The resulting vBOs exhibited vascular-like structures that were characterized at both transcriptional and protein levels, confirming the brain endothelial identity within the organoids. Functional assessment using drug permeability assays further demonstrated the relevance of the vascular network.

In a second step, we established a protocol to introduce a microglial population into vBOs. iPSCs were differentiated into microglia and co-cultured with vBOs generated from the same iPSC lineage. The resulting immunocompetent vBOs were characterized to quantify and spatially localize neuronal, endothelial, astrocyte and microglial markers. Stimulation with lipopolysaccharide (LPS) induced an inflammatory response, evidenced by increased expression of key microglial markers, including IBA1, and pro-inflammatory cytokines, thereby validating the functional relevance of the model. Altogether, these vascularized and immunocompetent cerebral organoids recapitulate key structural and functional features of the NVU and represent a promising three-dimensional human model for studying BBB biology and neuroinflammatory processes.

Stem cell-derived BBB and brain-on-a-chip platforms to study neurovascular adverse effects and drug penetration

Judit P. Vigh[1,2], Anna E. Kocsis[1,2], Ana R. Santa-Maria[1,3], Nóra Kucsápszky[1,2], Silvia Bolognin[4], Jens C. Schwamborn[4], András Kincses[1], Anikó Szecskó[1,2], Szilvia Veszelka[1], Mária Mészáros[1], Emese Bélai[1], Melinda Pirity[1], Sheila Sousa Gomes Fortes[5], Emanuel Carrilho[5], András Dér[1], Mária A. Deli[1,*], **Fruzsina R. Walter**[1,*]

[1]HUN-REN Biological Research Centre, Szeged, Hungary
[2]Doctoral School of Biology, University of Szeged, Szeged, Hungary
[3]Wyss Institute, Harvard University, Boston, MA, USA
[4]University of Luxembourg, Belvaux, Luxembourg
[5]Instituto de Química de São Carlos, Universidade de São Paulo, São Carlos SP, Brasil
**E-mail:* walter.fruzsina@brc.hu

Microfluidic chip devices allow more complex and physiological modelling of the central nervous system (CNS) and the blood-brain barrier (BBB). The BBB provides oxygen and nutrients for parenchyma and protects the brain from harmful effects. These protective mechanisms restrict the entry of pharmaceutical drugs into the brain limiting the treatment of CNS diseases. Stem cell-based technologies and human brain organoids provide a new platform to study diseases, adverse effects of psychoactive and clinically used drugs and agents, cellular interactions and drug uptake in a 3D setup.

We investigated the adverse effects of the psychoactive Δ9-tetrahydrocannabinol (THC), iodine containing clinically used contrast agent, iopamidol, and proinflammatory cytokines, as well as nanoparticle penetration using our complex, dynamic brain-on-a-chip model and a hydrogel-based BBB-on-a-chip.

The brain-on-a-chip model consists of a BBB part, co-culture of human stem cell derived endothelial cells and brain pericytes, integrated with human cortical or midbrain organoids differentiated from healthy donor iPSCs. The barrier integrity of the BBB model was examined in a dynamic setup by the measurement of impedance and permeability for 4 kDa FITC-dextran and Evans blue-labeled albumin markers. The morphology of brain endothelial cells was examined by immunostaining for claudin-5 tight junction protein and ZO-1 and β-catenin tight junction associated molecules. The model was used to measure the passage of targeted polypeptide nanocarriers across the BBB and their uptake into the organoids. Effect of pro-inflammatory cytokines was investigated by using a newly set up thrombin-fibrinogen mix-based hydrogel matrix.

THC treatment altered BBB integrity, morphology and viability of brain organoids. The hyperosmolar contrast agent iopamidol increased BBB permeability and damaged neuronal network of brain organoids. BBB-targeted nanoparticles carrying a fluorescent cargo crossed the BBB and entered the organoids effectively. Pro-inflammatory cytokines decreased cell viability in the hydrogel-based BBB-chip. In conclusion, the new BBB and brain-on-a-chip systems are valuable research tools for toxicological, pharmacological and pathological investigations of the CNS.

Acknowledgments: The project was supported by the National Research, Development and Innovation Office OTKA-K 143766 and ERA-NET-Neuron, B3Phrenia and NAP2022-I-6/2022 (to M.A.D.), NKKP Advanced Grant-153360 (to F.R.W.) and EKÖP-614-SZTE for J.P.V. and EKÖP-464-SZTE for A.E.K. Also, by the Hungarian Academy of Sciences Lendület „Momentum" Program (to F.R.W., LP2025-22/2025).

Sympathetic denervation attenuates pulmonary edema following experimental aneurysmal subarachnoid hemorrhage by protecting the pulmonary vascular endothelial glycocalyx

Nozomi Sasaki[1,2], Yusuke Egashira[1,2], Hideshi Okada[3], Masaki Kumagai[4], Hirofumi Matsubara[1], Yukiko Enomoto[1], Masamitsu Shimazawa[2], Hideaki Hara[2], and Tsuyoshi Izumo[1]

[1]*Department of Neurosurgery, Gifu University Graduate School of Medicine, Gifu, Japan*
[2]*Molecular pharmacology, Department of Biofunctional Evaluation, Gifu Pharmaceutical University, Gifu, Japan*
[3]*Department of Emergency and Disaster Medicine, Gifu, University Graduate School of Medicine, Gifu, Japan*
[4]*Department of Neurosurgery, Takayama Red Cross Hospital, Gifu, Japan*

Although the activation of the sympathetic nervous system (SNS) is associated with neurogenic pulmonary edema (NPE) following aneurysmal subarachnoid hemorrhage (SAH), pathological changes in the pulmonary capillary endothelium remain unclear. This study investigated whether SNS activation after SAH impairs the pulmonary endothelial glycocalyx (PEG) and results in NPE. Male ddy mice underwent prior surgical sympathetic blockade (superior cervical ganglionectomy; SCGx), and their lungs were evaluated after SAH. Lung water content and histopathology were evaluated in the sham, SAH, and SCGx + SAH groups. PEG was observed using scanning electron microscopy (SEM), and the degree of damage was quantified by fluorescent staining. Lung water content in the SAH group was significantly higher than that in the sham group (81.3% vs. 78.3%, $P < 0.01$). However, this was significantly attenuated in the SCGx + SAH group (79.7% vs. 81.3% vs. SAH, $P < 0.05$). Histopathology showed a similar trend. SEM revealed that a moss-like glycocalyx lined the endothelial lumen in sham mice. This structure was disrupted in the SAH mice, whereas the degree of shedding was mitigated in the SCGx + SAH mice. Fluorescence intensity in the SAH group was significantly lower than that in the sham group (14.5 vs 38.0; vs. sham, $P < 0.001$). However, this was significantly attenuated in the SCGx + SAH group (29.4 vs 14.5; vs. SAH, $P < 0.001$). NPE and PEG shedding following SAH was attenuated in desympathized lungs. Suppressing the SNS may be a therapeutic target for preventing SAH-induced NPE.

Central nervous system fibroblasts remodel the cerebrovascular basement membranes through osteopontin signaling in Alzheimer's disease

Kiersten Scott[1], Vasilia Kyriakopoulos[1], **Aki Urayama**[1]

[1]Department of Neurology, McGovern Medical School, University of Texas Health Science Center at Houston 6431 Fannin St, Houston, Texas 77030, USA

Background: Cerebrovascular basement membranes (vBMs) play a pivotal role in vascular fragility and the development of amyloid-beta (A□) aggregation on the vascular wall, leading to cerebral amyloid angiopathy (CAA). The resulting vascular leakage further remodels the vBM, creating a vicious cycle of cerebrovascular deterioration. Recent studies have found that secreted osteopontin (OPN) contributes to perivascular fibrosis. Using single-cell RNA sequencing (scRNAseq) and optical imaging approaches, we explored the potential role of pro-fibrotic central nervous system (CNS) fibroblasts in the remodeling of vBM with a focus on OPN signaling.

Methods: We performed scRNAseq in whole brain (minus cerebellum) blood vessel-enriched preparations in young (6 months) and aged (18 months) Tg2576 exhibiting type II CAA, and age-matched wild-type (WT) littermate mice. To identify translationally relevant targets, we also employed publicly available scRNAseq data sets from patients with AD, thereby comparing clinical AD and type II CAA mouse models. We conducted two-photon imaging to identify structural changes in fibrotic collagen layers surrounding the leptomeningeal blood vessels in Tg2576 mice compared to age-matched WT mice. Cortical and vascular amyloid deposition was imaged with methoxy-X04 (10 mg/kg, i.p.). Leptomeningeal vessels were labeled by tomato lectin AF649 (2%, 200 uL i.v.), and fibrotic collagen was labeled by second harmonic generation (SHG light at 405 nm wavelengths). Image analyses were performed by 3D-reconstructed volumetric image data sets with Arivis Vision 4D software.

Results: Through scRNAseq analyses, we found that OPN signaling was significantly upregulated in the brains of young Tg2576 mice predominantly seen in CNS fibroblasts. OPN was also upregulated in AD patient's brains. At the gene level, the interaction network from fibroblasts to microglia predicted dysregulated APP signaling, and dysregulated vBM interactions from fibroblast collagen to microglial integrin, including ITGA9, ITGB1, ITGAV, and ITGB5. Cell-cell interaction networks predicted that dysregulated fibroblast matrix components, including collagen, laminin, and fibronectin isoforms, interacted with astrocyte integrins and OPN receptor (CD44). These results suggest that regulation of the vBM through OPN signaling is multimodal, requiring the interplay of perivascular microglia, fibroblasts, and astrocytes. SHG imaging of leptomeningeal blood vessels in Tg2576 mice revealed increased fibrotic collagen layers and abnormal structural organization of the vasculature with aging.

Conclusion: The present study suggests that CNS fibroblasts interact with perivascular microglia and astrocytes through OPN signaling which mediates vBM remodeling in type II CAA model mice and patients with AD.

CLDN5-related neurological disease

Yosuke Hashimoto[1, 2], Gergő Porkoláb[2], Natalie Hudson[2], Chris Greene[2], Karine Poirier[3], Nathalie Boddaert[3, 5], Arnold Munnich[3, 4], Yasuo Uchida[1], Matthew Campbell[2]

[1]*Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan*
[2]*Smurfit Institute of Genetics, Trinity College Dublin, Dublin, Ireland*
[3]*Institut Imagine, Université Paris-Cité, Paris, France*
[4]*Clinical Genetics Department, Necker Hospital, AP-HP Paris Cité University, Paris, France*
[5]*Department of Pediatric Radiology, Hôpital Necker-Enfants malades, Paris, France*

Claudin-5 (CLDN-5) is an essential component of endothelial tight junctions that control the paracellular permeation of ions and solutes in the blood-brain barrier. We have identified a novel *de novo* missense mutation in *CLDN5* gene (Gly60Arg (G60R) mutation) from patients with alternating hemiplegia of childhood with brain calcification[1)]. As CLDN-5 is only expressed in endothelial cells among brain cells including neurons, astrocytes, or choroid plexus epithelial cells, the neurological abnormalities in patients with CLDN-5 G60R mutant or other CLDN-5 missense mutants[2)] can be envisaged as a disease of the blood-brain barrier. To reveal the pathogenic mechanism of this BBB-related disease, we have characterized the barrier function of mutated CLDN-5 and have developed a knock-in mouse carrying CLDN-5 G60R mutation. I would like to share recent our finding using this model mouse and future research that will be performed using instrument in our lab.

Sources:

1) Y. Hashimoto, K. Poirier, N. Boddaert, L. Hubert, M. Aubart, A. Kaminska, M. Alison, I. Desguerre, A. Munnich, M. Campbell. Recurrent *de novo* mutations in *CLDN5* induce an anion-selective blood–brain barrier and alternating hemiplegia. *Brain*, 145(10):3374–3382 (2022).
2) A.R. Deshwar, C. Cytrynbaum, H. Murthy, J. Zon, D. Chitayat, J. Volpatti, R. Newbury-Ecob, S. Ellard, H.L. Allen, E.P. Yu, R. Noche, et al., Variants in *CLDN5* cause a syndrome characterized by seizures, microcephaly and brain calcifications. *Brain*, 146(6):2285–2297 (2023).

Blood–brain barrier dysfunction in tick-borne encephalitis: NS1 as a potential antiviral and therapeutic target

M. Cizkova[1,3], V. Pranclova[1,3], E. Kotounova[1,3], M. Vancova[1], M. Davidkova[1], M. Dvorakova[1,3], L. Novotna[1,3], V. Hönig[1,2], **M. Palus**[1,2,4]

[1]*Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, CZ-37005 České Budějovice, Czech Republic*
[2]*Department of Virology, Veterinary Research Institute, Hudcova 70, CZ-62100 Brno, Czech Republic*
[3]*Faculty of Science, University of South Bohemia, Branišovská 31, CZ-37005 České Budějovice, Czech Republic*
[4]*Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5, CZ-62500 Brno, Czech Republic*

Tick-borne encephalitis virus (TBEV) is a neurotropic flavivirus causing severe central nervous system disease frequently accompanied by blood–brain barrier (BBB) dysfunction and long-term neurological sequelae. While neuronal infection represents a hallmark of disease, the mechanisms linking viral infection to BBB impairment remain incompletely understood and clinically unexplored.

Here, we investigated the contribution of neurovascular unit (NVU) cells and the viral non-structural protein 1 (NS1) to BBB dysfunction using in vitro human BBB models and in vivo mouse studies. TBEV productively infected endothelial cells, pericytes, astrocytes, and microglia with cell-type-specific kinetics. Pericytes supported non-cytopathic infection and responded with strong secretion of inflammatory and chemotactic mediators, suggesting their role in shaping the inflammatory milieu at the BBB. Microglia sustained long-term infection and displayed strain-dependent immune activation, indicating their involvement in persistent neuroinflammation.

Importantly, TBEV crossed intact endothelial monolayers without immediate tight junction disruption. NS1 significantly increased endothelial permeability and disrupted the endothelial glycocalyx, identifying NS1 as a key mediator of early BBB dysfunction. Targeting NS1 using monoclonal antibodies or immunization strategies partially protected mice and prolonged survival, supporting its translational relevance.

Together, these findings highlight NS1-driven BBB impairment as a mechanism of TBEV neuropathogenesis and position NS1 as a promising antiviral and therapeutic target bridging basic and clinical research.

Microglia modulate neurovascular responses under systemic inflammatory conditions

Ádám Dénes[1]

[1]Laboratory of Neuroimmunology, HUN-REN KOKI, Budapest, Hungary

Microglial phenotypes are altered in common brain diseases, but how microglial modulation of neurovascular processes changes in diverse disease states, is not well understood. We have identified novel purinergic interactions at specified areas of neuronal somata that are maintained in accordance with changes in neuronal activity and metabolic states. Microglia also shape vascular responses via similar, compartment-specific actions, contributing to the regulation of cerebral blood flow, neurovascular coupling and cerebral hypoperfusion. In the inflamed brain, altered microglia-neurovascular interactions are associated with perfusion changes and modulation of central leukocyte recruitment. Surprisingly, microglial interactions with different cell types of the neurovascular unit not only change markedly under systemic inflammatory conditions, but these changes occur differently in given arterial and venous vascular beds. The translational impact of these cell-cell interactions is suggested by largely similar molecular fingerprints observed in mouse and human brains, which is being systematically assessed in different neurological conditons to identify the key molecules that contribute to altered neurovascular modulation by microglia across disease states. Understanding the molecular mechanisms of microglia-neurovascular interactions is likely to help the identification of novel therapeutic targets in common neurological disorders.

Brain shuttle target expression levels vary by individual, not by brain region, disease, age, or gender

Ana R. Santa-Maria[1], Sanjid Shahriar[1], Vasanth Chandrasekhar[1], Emma Luteijn[1], Robin Horber[1], Samuel Hornstein[1,2], Sasha Tkachev[3], Tyler Levy[3], Ivan Gregoretti[3], Claire Simpson[3], Majd Ariss[3], Vilas Menon[4], Donald Ingber[1,5,6], James Gorman[1]

[1]*Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA 02215, USA*
[2]*Sidney Kimmel Medical College, Thomas Jefferson University Hospital, Philadelphia, PA 19107, USA*
[3]*Cell Signaling Technology, Danvers, MA 01923, USA*
[4]*Columbia University Irving Medical Center, New York, NY 10032, USA*
[5]*Vascular Biology Program and Department of Surgery, Boston Children's Hospital and Harvard Medical School, Boston, MA 02115, USA*
[6]*Harvard John Paulson School of Engineering and Applied Sciences, Cambridge, MA 02134 USA*

Identification of surface protein targets on brain microvascular endothelial cells (BMECs) that enhance molecular transport across the blood–brain barrier (BBB) has facilitated the development of brain shuttles that bind to these transporters and thereby enhance delivery of large molecule drugs and biologics to brain tissue. However, the extent to which brain shuttle target expression varies between brain regions, disease states, demographic groups, and individuals remains unclear. Here, we analyzed variation in gene and protein expression levels of canonical brain shuttle targets in isolated human BMECs and human brain microvessels from large, diverse cohorts using single-cell and single-nucleus transcriptomics and quantitative proteomics. Our results demonstrate that global cell surface protein expression and individual abundances of eleven BBB shuttle targets are remarkably stable between brain regions, genders, ages, and major neurodegenerative conditions, including Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis. Notably, regional heterogeneity observed in rodent models, such as for transferrin receptor, was not detected in human tissues, and disease status had minimal impact on brain shuttle target expression. Age-associated changes in target expression were modest. The most significant source of variability in brain shuttle target abundance was inter-individual differences, with levels differing by up to an order of magnitude between individuals in each demographic or clinical group. These findings indicate that canonical brain shuttle target expression is principally determined at the individual level and that patient-specific quantitative assessment of brain shuttle target abundance may be essential for optimization of drug delivery to the brain using brain shuttles.

A proof of concept: a delivery system to transport antimicrobial peptides across BBB against neuropathogens

M. Bhide[1,2,*]

[1]*Laboratory of Biomedical Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovakia*
[2]*Institute of Neuroimmunology, Slovak Academy of Sciences v. v. i., Dúbravská cesta 9, 845 10 Bratislava, Slovakia*
**E-mail: bhidemangesh@gmail.com*

Infectious diseases affecting the central nervous system (CNS) remain an important source of morbidity and mortality. A major obstacle for curing brain diseases is the blood-brain barrier (BBB), which impedes therapeutic agents from reaching the brain and targeting the related pathogens. The aim was to develop a drug delivery system derived from antimicrobial peptide and the CNS homing peptide. We developed antiviral and anti-borrelial peptides using combinatorial phage display and tested their activity and cell toxicity. The best peptides were further fused with CNS-homing peptides (such as Angiopep-2) to increase their biodistribution in the brain parenchyma. In parallel, we also developed the novel-CNS homing peptide derived from OspA protein of the neuroinvasive *Borrelia* and fused with the antimicrobial peptide. In this way, we successfully produced fusion peptides against Tick-borne encephalitis virus, SARS-CoV-2 and neuroinvasive *Borrelia*. Development of all fusion peptides, their antimicrobial activity, and crossing across the BBB will be presented in the BBB-mini symposium. We expect to generate clinically useful pilot results for the best-performing candidates for future translation and, at the same time, research data of general scientific interest useful to the broad scientific community.

Acknowledgments: The research was funded by grants VEGA 1/0381/23 and APVV-22-0084.

Expression of alpha smooth muscle actin decreases with ageing and increases upon lumen obstruction in mouse brain pericytes

Fanni Győri[1,2], Ádám Mészáros[1], Mónika Krecsmarik[1], Kinga Molnár[1], Cornel Balta[3], Anca Hermenean[3], Attila E. Farkas[1], István A. Krizbai[1,3,*], Imola Wilhelm[1,3,*]

[1]*Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary*
[2]*Theoretical Medicine Doctoral School, University of Szeged, Szeged, Hungary*
[3]*"Aurel Ardelean" Institute of Life Sciences, Vasile Goldiş Western University, Arad, Romania*

Cerebral pericytes are mural cells covering brain microvessels, organized as ensheathing, mesh and thin-strand pericytes. These latter two, together called capillary pericytes, have low levels of alpha smooth muscle actin (α-SMA), regulating basal vascular tone and applying a slow influence on cerebral blood flow.

Pericytes are subject to alterations in ageing which may be even more pronounced in age-related pathologies, including microinfarcts, which usually affect a large number of vessels in the ageing brain. We modelled this condition by injecting 10 μm-size microspheres into the circulation of mice resulting in the occlusion of capillaries covered by ensheathing and mesh pericytes.

We observed that α-SMA and Acta2, the gene encoding it, as well as TGF-β1/Tgfb1, the major regulator of α-SMA, decreased during ageing in cerebral microvessels. In the vicinity of the microspheres stalled in the capillaries, expression of α-SMA increased significantly in both ensheathing and especially in mesh pericytes, both in young (2 to 3 months of age) and old (24 months of age) mice. On the other hand, γ-actin was detected in endothelial cells, but not in pericytes, and decreased in microvessels of microsphere-containing hemispheres.

Altogether, our data show that obstruction of cerebral microvessels increases α-SMA expression in pericytes in both age groups, but this does not compensate for the lower expression of the contractile protein in old animals. Increased α-SMA expression may lead to constriction of the obstructed vessels probably aggravating flow heterogeneity in the aged brain.

Brain diffusion of Tau protein and its transport across the blood–CSF barrier in neurodegeneration

Petra Majerova[1], Krutika Khiratkar[1], Matthew Rubin[2,3], Martin Cente[1], Sabina Hrabetova[2], Andrej Kovac[1]

[1]Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia
[2]Department of Cell Biology, SUNY Downstate Health Sciences University, Brooklyn, New York, USA
[3]Martin J. Whitman School of Management, Syracuse University, Syracuse, New York, USA

Tauopathies are a group of neurodegenerative disorders, including the most prevalent form, Alzheimer's disease, characterized by abnormal aggregation of tau protein into neurofibrillary tangles. Since tau is widely used as a biomarker for the diagnosis and monitoring of Alzheimer's disease progression, understanding its diffusion and influx–efflux dynamics is important. Here, we investigated the behavior of tau protein in the central nervous system (CNS) and its transport across the blood–cerebrospinal fluid barrier (BCSFB). Using the integrative optical imaging method in agarose gel and acute mouse brain slices, we analyzed the extracellular diffusion properties of tau proteins in the brain. We found that the diffusion permeability of tau was comparable in the entorhinal cortex (EC) and prefrontal cortex (PFC), two brain regions commonly affected in tauopathies. However, extracellular diffusion of tau in the EC was slower compared with other proteins and dextrans, suggesting that charge-based interactions between tau and negatively charged components of the extracellular matrix may retard its diffusion. Furthermore, we systematically characterized the ability of several tau fragments (aa 1–146, aa 121–227, and aa 151–391) to cross the BCSFB. For the experiments we used an established in vitro model of the BCSFB obtained by culturing primary epithelial cells isolated from rat choroid plexus. We observed that N-terminal fragments of tau are efficiently transported from the brain to the blood, whereas C-terminal tau proteins show limited transport. Our findings indicate that tau diffusion within the CNS and its transport across the BCSFB may influence its distribution and clearance, potentially contributing to the pathophysiology of tauopathies such as Alzheimer's disease.

Acknowledgments: APVV-22-0313, APVV-21-0321

The histone deacetylase inhibitor suberoylanilide hydroxamic acid promotes blood-brain barrier protection during reperfusion in a cell culture model of ischemic stroke

Anikó Szecskó[1,2,*], Koppány Párdi[1,*], **Zuhao Cui[1,2,*]**, Gergő Porkoláb[1,+], Zsófia Hoyk[1], Csilla Kovács[1], Nárcisz M. Cser[1], László Dér[3], Krisztina Nagy[3], Mária A. Deli[1], Szilvia Veszelka[1]

1Biological Barriers Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary
2Doctoral School of Biology, University of Szeged, Szeged, Hungary
3Biophotonics and Biomicrofluidics Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary
**These authors contributed equally to this work.*

Introduction: Ischemic stroke, characterized by acute cerebral vascular occlusion and subsequent blood-brain barrier (BBB) breakdown, remains a major clinical challenge due to reperfusion injury and limited therapeutic options. Histone deacetylase (HDAC) inhibitors have emerged as potential neuroprotective agents; however, their role in preserving BBB integrity is not fully understood. This study aimed to investigate the effect of the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) on BBB function in an in vitro model of ischemic stroke and explore its potential for clinical repurposing.

Method: The effects of SAHA were tested on human co-cultured BBB model under normoxia, and after 6 hours oxygen-glucose deprivation (OGD) following 24 hours reperfusion (OGD/R) conditions.

Results: Our results show that after OGD, SAHA is able to prevent the BBB functions by increasing the resistance, and reducing the permeability of marker molecules across the BBB. RNA-seq analyses showed that SAHA decreased the expression of genes involved in cell proliferation and increased the expression of glycocalyx related genes. Furthermore, SAHA enhanced the expression of genes of basement membrane components. Gene Set Enrichment Analysis (GSEA) further revealed significant enrichment of β-catenin independent Wnt signaling pathways, which are known to play an important role in cytoskeletal rearrangement and cellular structural organization. Morphological analysis confirmed these transcriptomic findings, showing that SAHA treatment led to a more elongated and differentiated brain endothelial phenotype, consistent with enhanced barrier stability and endothelial maturation.

Conclusions: We demonstrated that the HDAC inhibitor SAHA is capable of protecting brain endothelial cells from ischemic damage by strengthening BBB functions in a cell culture model. Our study investigated in depth the molecular mechanisms underlying SAHA's protective effects on brain endothelial cells through gene expression profiling, functional assays, and metabolomic analysis. Based on our results, SAHA may represent a potential therapeutic strategy for the treatment of ischemic stroke by preserving BBB integrity and promoting endothelial stabilization. The observed morphological changes and upregulation of basement membrane components suggest that SAHA may improve endothelial structural organization and cell-matrix interactions, but further studies are needed to clarify this.

Acknowledgments: A.S. was supported by Gedeon Richter Talentum Foundation. S.V. was supported by the Young Researcher Excellence Program (FK 143233) by National Research, Development and Innovation Office of Hungary. G.P. was supported by the National Academy of Scientist Education (FEIF/646-4/2021-ITM_SZERZ).

POSTER ABSTRACTS

Poster 1

Bidirectional communication between brain metastatic cells and cells of the neurovascular unit

Rabiya Bano[1], Csilla Fazakas[1], Adél Lüvi[1], Maryam Naeem[1], Kinga Molnár[1], Attila E. Farkas[1], István Krizbai[1], Imola Wilhelm[1]

[1]*HUN-REN Biological Research Centre, Szeged, Hungary*

Brain metastases are fatal complications of certain tumour types, including triple negative breast cancer. During development of cerebral secondary tumours, transfer of signals between tumour cells and cells of the neurovascular unit is crucial in shaping the tumour microenvironment, making it more permissive for the cancer cells. In our experiments, we aimed to understand the pathways of information transmission between metastatic cells and cerebrovascular cells, including pericytes and endothelial cells.

We first assessed communication from brain cells towards the metastatic cells. In brain pericyte–breast cancer cell co-cultures, we observed formation of tunnelling nanotubes (TNTs), which interconnected the two cell types, and which channelled mitochondria from pericytes to the tumour cells. However, we could also find pericyte-derived mitochondria in breast cancer cells having no apparent TNT-connection to pericytes. In order to prove mitochondrial transfer through the culture medium, we seeded mouse breast cancer cells and human pericytes in a common culture medium, the two cell types being physically separated. Using species-specific primers and PCR, we detected expression of human mitochondrial (but not genomic) DNA in the mouse tumour cells. Similarly, when mouse brain pericytes were cultured with human triple negative breast cancer cells in common culture medium, but preventing direct contact between the two cell types, we showed the presence of mouse mitochondrial DNA, but not of mouse genomic DNA in the human tumour cells. Using a similar setup, we demonstrated transfer of mitochondrial DNA from brain endothelial cells to metastatic cells as well, in the absence of direct contact, possibly through extracellular vesicles (EVs).

To understand which brain cells are the main communication targets of metastatic breast cancer cells, we used Ai14 reporter mice, which express robust tdTomato fluorescence following Cre-mediated recombination. As a source of the Cre recombinase, we used engineered breast cancer cells, in which Cre expression was confirmed both in the cells and in the released EVs. Using this approach, we have shown both in vitro and in vivo, that brain endothelial cells are the main targets of the tumour cells, followed in number by pericytes.

In conclusion, we have demonstrated the bidirectional communication between metastatic cells and cells of the blood-brain barrier in the presence and absence of direct contacts. Mitochondrial transfer might be one of the mechanisms through which the host cells protect tumour cells in the brain.

Poster 2

Tick-borne encephalitis virus and its NS1 protein disrupt endothelial monolayer integrity and contribute to endothelial glycocalyx disruption

Monika Čížková[1,2], Marika Davídková[1], Markéta Dvořáková[1], Hana Sehadová[2,3], Veronika Prančlová[1,2], Eliška Kotounová[1,2], Martin Palus[1,4,5]

[1]Biology Centre CAS, Laboratory of Arbovirology, Ceske Budejovice, Czech Republic
[2]University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Republic
[3]Biology Centre CAS, Laboratory of Microscopy and Histology, Ceske Budejovice, Czech Republic
[4]Veterinary Research Institute, Department of Virology, Brno, Czech Republic
[5]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

Tick-borne encephalitis virus (TBEV) is a member of the genus Orthoflavivirus. During TBEV infection, disruption of the blood–brain barrier (BBB) has been observed. The BBB plays a key role in protecting the central nervous system (CNS). This protective function arises from its specialized cellular architecture, formed by the neurovascular unit (NVU), which includes endothelial cells, astrocytes, and pericytes. One of the factors contributing to BBB integrity is the endothelial glycocalyx. This study investigates the ability of the TBEV nonstructural protein 1 (NS1) to compromise BBB integrity and alter the endothelial glycocalyx.

We used an *in vitro* Transwell system to mimic the BBB. Human primary brain microvascular endothelial cells were seeded either alone or in co-culture with astrocytes and pericytes. Endothelial integrity was assessed by measuring transendothelial electrical resistance (TEER). The endothelial glycocalyx was examined by immunofluorescence staining and quantified by mean fluorescence intensity (MFI).

Exposure to TBEV NS1 significantly disrupted endothelial monolayer integrity in both models. In co-culture models, this disruption occurred regardless of whether NS1 was applied to the luminal or abluminal side of the Transwell system. Immunofluorescence analysis revealed a reduction in the glycocalyx and activation of enzymes responsible for glycocalyx degradation due to NS1 treatment. Moreover, TBEV infection also disrupted endothelial monolayer integrity, and productive viral replication was observed.

Our findings demonstrate that both TBEV NS1 and TBEV disrupt endothelial monolayer integrity and that NS1 can modify the endothelial glycocalyx. These results may help explain BBB disruption during TBEV infection and suggest potential therapeutic targets for TBEV-associated disease.

Poster 3

Investigation of the effects of antidepressant compounds from traditional chinese medicine on brain endothelial cells, and their permeability across the blood-brain barrier: a China-Hungary collaborative study

Zuhao Cui[1,2], Ana Martins[1], Aniko Szecsko[1,2], Tivadar Kiss[3], Andrea Vasas[3], Fruzsina R. Walter[1], Szilvia Veszelka[1], Attila Hunyadi[3], Gang Chen[4], Maria A. Deli[1]

[1]*Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary*
[2]*Doctoral School of Biology, University of Szeged, Szeged, Hungary*
[3]*Institute of Pharmacognosy, University of Szeged, Szeged, Hungary*
[4]*Interdisciplinary Institute for Personalized Medicine in Brain Disorders, and Guangdong-Hong Kong-Macao joint Laboratory of Traditional Chinese Medicine, Jinan University, Guangzhou, China*

Major depressive disorder remains one of the most prevalent psychiatric illnesses worldwide. Developing rapid-acting antidepressant therapies is a critical unmet need. The blood-brain barrier (BBB) plays key role in maintaining central nervous system homeostasis by tightly regulating the entry of molecules from systemic circulation to the brain. Its selectivity limits the delivery of many therapeutic agents to the brain.

In this collaborative project between Jinan University and HUN-REN Biological Research Centre, Szeged, we aim to explore the BBB permeability and pharmacological effects of four active compounds isolated from traditional Chinese herbal medicine: shanzhiside methyl ester, geniposide, paeonol, and ferulic acid, which presumably play important roles in antidepressant effects of the Jiawei-Xiaoyao and Yueju formulas. Previous work has shown that these compounds and/or their combinations may contribute to rapid antidepressant-like effects through modulation of PACAP signaling and related neurobiological pathways.

A co-culture human BBB model, consisting of stem cell derived endothelial cells and brain pericytes, is used to reproduce physiologically BBB properties. Cell effects were monitored by real-time impedance measurements, while compound permeability and quantification is currently being investigated by using HPLC. The results aim to clarify BBB transport mechanisms and support the development of herbal antidepressants with improved brain bioavailability.

Poster 4

Capillary pericytes regulate vascular tone and local blood flow in inflammation

Tamás Dudás[1,2], Ádám Mészáros[1], Kinga Mészáros-Molnár[1], Attila E. Farkas[1], Imola Wilhelm[1], István Krizbai[1]

[1]*Biological Research Centre, Szeged, Institute of Biophysics*
[2]*Doctoral School of Experimental and Preventive Medicine, University of Szeged, Szeged, Hungary*

Pericytes are the only contractile cells in cerebral capillaries. However, their role in the regulation of capillary diameter, microvascular tone and local cerebral blood flow is far from being completely understood. Furthermore, a large number of CNS disorders is accompanied by inflammatory processes. Therefore, in our present study, we investigated the role of pericytes in the regulation of cerebral blood flow during inflammatory conditions and their contractility.

Using primary human pericytes in an in vitro collagen contraction assay, we could demonstrate that TNFalpha, IL-6 and CCL2 induce a significant pericyte contraction. In order to prove that inflammatory mediators have similar effects in vivo, we used two photon microscopy in mice with labelled pericytes. Inflammatory mediators were administered either in the vicinity of identified pericytes using microinjection techniques or with systemic administration using peripheral cannulation techniques under continuous monitoring . TNF-alpha induced a slow but significant reduction of capillary diameter and pericyte cell body. In addition, using line scan technology, we could show a decrease in red blood cell velocity and a reduction in the number of red blood cells passing the capillary segment in the neighbourhood of the injection. Interestingly, TNF-alpha coming from the periphery induced vasodilation while contracting capillary pericytes. Moreover, the vasodilation was accompained by significantly increased red blood cell flux.

Our findings strongly suggest that pericytes may have an important role in the regulation of cerebral blood flow under inflammatory conditions. Moreover, the mechanism of regulation might differ depending on where the signal comes from (CNS or periphery).

Acknowledgements: This work was supported by the National Research, Development and Innovation Office of Hungary (K135425), the National Brain Research Program NAP 3.0 of the Hungarian Academy of Sciences and the Gedeon Richter Talentum Foundation in framework of Gedeon Richter Excellence PhD Scholarship of Gedeon Richter.

Poster 5

Extracellular vesicles from triple negative breast cancer cells disrupt the blood–brain barrier via miR-146a-5p- and TGF-β1-mediated downregulation of endothelial Paqr5

Csilla Fazakas[1], Attila G. Végh[1], Tamás Dudás[1,2], Dorina Varga[1], Adél Lüvi[1], Mónika Krecsmarik[1], András Dér[1], Attila E. Farkas[1], István A. Krizbai[1,3,4,*], Imola Wilhelm[1,4,*,#]

[1]*Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary*
[2]*Doctoral School of Experimental and Preventive Medicine, University of Szeged, Szeged, Hungary*
[3]*Department of Cell Biology and Molecular Medicine, University of Szeged, Szeged, Hungary*
[4]*"Aurel Ardelean" Institute of Life Sciences, Vasile Goldiş Western University, Arad, Romania*
**Authors contributed equally*
[#]*Corresponding author e-mail: wilhelm.imola@brc.hu*

Key words: blood–brain barrier, brain metastases, claudin-5, extracellular vesicles, membrane progesterone receptor, *Paqr5*/mPRγ, triple negative breast cancer

The molecular mechanisms driving brain metastasis formation, including cancer cell migration across the blood–brain barrier (BBB), are not yet fully defined. Using a highly relevant *in vitro* mouse model system, human cell cultures, and a mouse brain metastasis model, we sought to elucidate how triple negative breast cancer (TNBC) cells – one of the major sources of secondary brain tumours – and the extracellular vesicles (EVs) they release modulate the BBB-forming endothelium to render it more permissive for tumour cell entry into the brain. We observed that EVs derived from TNBC cells are taken up by cerebral endothelial cells, where they induce miR-146a-5p- and TGF-β1-mediated downregulation of *Paqr5*/mPRγ, a membrane progesterone receptor. This, in turn, leads to disruption of interendothelial tight junctions, particularly through repression of claudin-5 expression. As a consequence of BBB opening, cancer cells migrate in greater numbers across brain endothelial monolayers with reduced Paqr5 expression. In conclusion, we identify a novel mechanism by which TNBC-derived EVs compromise BBB integrity, thereby facilitating transendothelial migration of cancer cells and promoting brain metastasis development.

Poster 6

An integrated microphysiological platform for barrier modeling and whole-organoid imaging

Luíza Santa Brígida de Barros Góes[1,2,3], László Dér[1], Emese Bélai[4], Pirity Melinda Katalin[4], Sándor Valkai[1], András Dér[1], Mária A. Deli[1], Fruzsina R. Walter[1,*], Emanuel Carrilho[2,3,*]

[1]*Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary*
[2]*Instituto de Química de São Carlos, Universidade de São Paulo 400, Brazil*
[3]*Instituto Nacional de Ciência e Tecnologia de Bioanalítica Lauro Kubota – INCTBio-LK Campinas SP, Brazil*
[4]*Institute of Genetics, HUN-REN Biological Research Centre, Szeged, Hungary*
**Authors contributed equally*

Traditional *in vitro* models, such as two-dimensional cell cultures, often fail to capture the complexity of living tissues and their interactions. In response, advanced *in vitro* approaches have been developed to study three-dimensional (3D) tissue organization, functional biological barriers, and dynamic cellular processes. These approaches provide complementary perspectives on tissue behavior, enabling more detailed investigation of organ-level interactions, barrier functions, and multicellular dynamics under controlled experimental conditions.

Here, we present an experimental framework of a low-cost multi-organ microphysiological system (MPS); modular 3D-printed inserts for barrier formation; and tissue clearing approaches for whole-organoid imaging. The MPS was fabricated from polymethyl methacrylate (PMMA) sheets of varying thickness assembled using biocompatible double-sided adhesive and a thin polydimethylsiloxane (PDMS) gasket, enabling reversible assembly while maintaining leak-proof operation. The device incorporates three circular chambers, each divided into upper and lower compartments by porous membranes, allowing endothelial or epithelial barrier formation under independently controlled perfusion conditions. Organoid compartments can be supplied with either a shared medium or compartment-specific media through separate inlets.

To test compatible cell culture porous membranes, custom cell culture inserts were designed using computer-aided design (AutoCAD) software and 3D printed by using a biocompatible, autoclavable resin. Porous membranes were integrated into the inserts using a silicone-based biocompatible adhesive, generating interchangeable modules compatible with different membrane types. Barrier performance was explored using permeability assays and immunofluorescence analyses in distinct biological barrier models. In parallel, to enable volumetric imaging of organoids cultured within this integrated system, four tissue clearing strategies: CUBIC, Binaree, sucrose- and fructose-based methods were evaluated in midbrain and cortical organoids.

The proposed platform enables the combined use of modular barrier inserts, controlled perfusion, and downstream tissue clearing within a single experimental workflow. This work establishes an adaptable and accessible framework for multi-organ *in vitro* studies that integrates device engineering, barrier modeling, and whole-organoid imaging. The platform provides a basis for systematic optimization and future functional validation of biological barriers and organoid interactions, supporting its application in advanced disease modeling and translational research.

Acknowledgement: The project was supported by the Hungarian National Research Development and Innovation Office of Hungary (K143766, 2024-1.2.2-ERA_NET-2024-00018 and ADV153360 for F.R.W.). F.R.W. is also funded by the Lendület "Momentum" Research Grant (LP2025-22/2025). Also supported by the National Council for Scientific and Technological Development (CNPq), process no. 200562/2025-8.

Poster 7

Microfluidic synthesis and physicochemical characterization of petox-pcl nanoparticles

S. Göksever[1,2,*], B. Küçüktürkmen[1], UC. Oz[1], A. Hunyadi[3,4], A. Bozkır[1]

[1]*Ankara University, Department of Pharmaceutical Technology, Ankara, Turkey*
[2]*Ankara University, Institute of Health Sciences, Ankara, Turkey*
[3]*Institute of Pharmacognosy, University of Szeged, Szeged, Hungary*
[4]*HUN-REN-SZTE Biologically Active Natural Products Research Group, Szeged, Hungary*
**E-mail: skurter@ankara.edu.tr*

Introduction:

Poly(2-ethyl-2-oxazoline) (PEtOx) is an FDA-approved polymer recognized for its high biocompatibility and prolonged circulation properties. When combined with polycaprolactone (PCL), it forms stable nanocarriers (1) suitable for the delivery of neuroprotective agents such as our natural lead compound (CS). As particle size is a critical parameter for blood–brain barrier (BBB) transport, microfluidic systems offer a strategic advantage by enabling precise control over nanoparticle production (2). In this study, CS-loaded PEtOx-PCL nanoparticles were synthesized using a bifurcating microfluidic mixer, and their physicochemical properties were investigated.

Materials and Methods:

PEtOx-PCL nanoparticles were synthesized using a bifurcating mixer design. The effects of polymer concentration, flow rate ratio (FRR), and total flow rate (TFR) on the physicochemical properties of the nanoparticles were investigated. The physicochemical properties of the obtained nanoparticles (hydrodynamic diameter, PDI, and zeta potential) were analyzed via Dynamic Light Scattering (DLS).

Results:

The bifurcating mixer produced nanoparticles with hydrodynamic diameters below 100 nm, PDI<0.3, and a zeta potential of approximately −15 mV.

Conclusions:

In conclusion, the bifurcating mixer enabled the reproducible production of PEtOx-PCL nanoparticles suitable for BBB transport. This design serves as a robust platform for the development of nanoparticles, offering precise control over critical physicochemical parameters. Optimization studies will continue using microfluidic devices with different materials and mixing designs to further improve the production process.

Acknowledgement: This study was funded by the NRDIO–TÜBİTAK Bilateral Project (222N021).

References

1. Ozkose, U.U., Yilmaz, O., Alpturk, O. (2019). Synthesis of poly(2-ethyl-2-oxazoline)-b-poly(ε-caprolactone) conjugates by a new modular strategy. Polymer Bulletin, 77(11), 5647-5662.
2. Bezelya, A., Küçüktürkmen, B., Bozkır, A. (2023). Microfluidic Devices for Precision Nanoparticle Production. Micro, 3(4), 822-866.

Poster 8

Investigation of a pentapeptide carrier on a culture model of the blood-brain barrier

Ilona Gróf[1], Alexandra Bocsik[1], Milán Szántó[1], Enikő Szabó[2], Imre Norbert[3], Tamás Martinek[3], Mária A. Deli[1]

[1]*Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary*
[2]*Institute of Genetics, HUN-REN Biological Research Centre, Szeged, Hungary*
[3]*Department of Medical Chemistry University of Szeged, Szeged, Hungary*

The blood-brain barrier (BBB) makes the treatment of many central nervous system related diseases difficult, as it blocks the delivery of biopharmaceutics at therapeutically relevant concentrations. Therefore, targeted delivery of protein drugs to the intracellular space and through BBB is an actively investigated area. One of the promising candidates are the glycan-recognizing peptides. Our research group has previously shown that the galectin-1- derived WYKYW pentapeptide binds to GM1 ganglioside on the cell surface at nanomolar concentrations and is capable of delivering large proteins into the intracellular space via lipid-raft mediated/caveolar endocytosis without being trapped in lysosomes (Imre et al., 2020). The WYKYW peptide is small and does not influence the viability of cells, thus it is a promising candidate as a carrier for proteins and nanoparticles to cross BBB. Since no data are available on whether WYKYW is suitable for delivering large cargoes into cells or for transfer through cell layers, our aim was to compare the cellular entry of the peptide complexes into cells and their penetration across the culture model of the BBB. Human brain endothelial cells were cultured on glass bottom dishes to investigate the intracellular localization of the WYKYW-protein and WYKYW-nanoparticle complexes by confocal microscopy. For the transcytosis experiment endothelial cells were co-cultured on cell culture inserts with brain pericytes. After the permeability experiment the immunostaining of intercellular junction proteins was performed. We observed that the peptide-protein and peptide-nanoparticle complexes entered into the brain endothelial cells and localized in the cytoplasm. Image analysis showed high colocalization of the WYKYW targeted nanoparticles with the endoplasmic reticulum in the brain endothelial cells. We have shown that the peptide did not enhance the penetration of cargoes through the cell layers. The peptide treatment did not change the staining pattern of intercellular junctions of endothelial cells, showing that the tightness of the barrier was not compromised. Our results suggest that the WYKYW pentapeptide is suitable for delivering large proteins and nanoparticles into brain endothelial cells through a specific endocytotic pathway, but does not enhance the transcellular passage of cargoes.

Poster 9

A versatile brain-on-a-chip system to study pathological conditions of the central nervous system

Anna E. Kocsis[1,2], Judit P. Vigh[1,2], Ana R. Santa-Maria[1,3], Nóra Kucsápszky[1,2], Silvia Bolognin[4], Jens C. Schwamborn[4], András Kincses[1], Anikó Szecskó[1,2], Szilvia Veszelka[1], Mária Mészáros[1], Emese Bélai[1], Melinda Pirity[1], András Dér[1], Fruzsina R. Walter[1,*], Mária A. Deli[1,*]

[1]*HUN-REN Biological Research Centre, Szeged, Hungary*
[2]*Doctoral School of Biology, University of Szeged, Szeged, Hungary*
[3]*Wyss Institute, Harvard University, Boston, MA, USA*
[4]*University of Luxembourg, Belvaux, Luxembourg*

Cerebral capillaries forming the blood-brain barrier (BBB) maintain brain homeostasis while restricting the entry of harmful substances and drugs into the brain. Microfluidic chip devices allow complex and physiological modelling of the BBB. Stem cell-based technologies and human brain organoids provide a new platform to study diseases, cellular interactions and drug uptake in a 3D setup.

Our aim was to study brain pathologies and BBB disruption using our dynamic brain-on-a-chip system. This device integrates a human stem cell-based BBB model with brain organoids. Human stem cell derived endothelial cells and brain pericytes were co-cultured to establish the BBB model. Human cortical organoids were differentiated from healthy donor iPSCs.

The barrier integrity of the BBB model was investigated after tetrahydrocannabinol (THC) treatment in a dynamic setup by the measurement of impedance and permeability for fluorescent markers. The morphology of brain endothelial cells was examined by immunostaining for tight junction associated proteins. THC treatment decreased the BBB integrity and altered the morphology of brain organoids. This brain-on-a-chip system can be a valuable research tool for toxicological, pharmacological and pathological investigations.

Acknowledgements: The project was supported by the Hungarian National Research Development and Innovation Office of Hungary (FK137808 for A.M.; K143766, 2024-1.2.2-ERA_NET-2024-00018 and ADV153360 for F.R.W.). F.R.W. is also funded by the Lendület "Momentum" Research Grant (LP2025-22/2025) and the Grant for Researchers Raising Small Children (56/2/2025/KP) program by the Hungarian Academy of Sciences, by the University Research Scholarship Programme (EKÖP-464-SZTE), which is a sholarship of the Ministry of Culture and Innovation funded by the National Research, Development and Innovation Office, and by the Richter Gedeon Talentum Foundation Research Grant.

Poster 10

Translational research aimed at improving heatstroke diagnosis

Andrej Kovac[1,*], Kazuyuki Miyamoto[2], Dorothy Wasike[1], Petra Majerova[1]

[1]Institute of Neuroimmunology, SAS, Bratislava, Slovakia
[2]Showa University, Department of Emergency and Disaster Medicine, Shinagawa-ku, Tokio, Japan

Global warming is expected to increase the frequency of natural disasters such as wildfires and storms, and, more directly, the incidence of heat-related illnesses. Among these, heatstroke (HS) is the most severe form. HS is characterized by a core body temperature exceeding 40 °C accompanied by central nervous system (CNS) dysfunction, and is often complicated by multi-organ failure involving renal, hepatic, and hemostatic systems.
In the present study, we established an animal model of HS using male C57BL/6J mice aged 20–24 weeks. The mice were exposed to a temperature of 42 °C and constant humidity (40 ± 15 %) for 45 minutes. Following exposure, plasma and brain tissue samples were collected for proteomic analysis.Peptide samples were separated on a nanoEase HSS T3 C18 analytical column and analyzed on a Synapt G2-Si quadrupole time-of-flight mass spectrometer. Data processing was performed using Progenesis QI 4.0. In addition, we conducted a targeted analysis of potential biomarkers associated with inflammation and synaptic dysfunction reported in HS. Using the ultrasensitive Single Molecule Array (SIMOA) system, we quantified low-abundance proteins in plasma, including neurofilament light chain (NfL). Together, these analyses allowed us to identify proteins potentially involved in modulating molecular processes related to heatstroke pathophysiology.

Acknowledgements:
EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V03- 00086, APVV-21-0321, VEGA 02/0075/24.

Poster 11

Blood-brain barrier disruption in acute pancreatitis: a clinical and cell-culture study

Nóra Kucsápszky[1,2,3], **Ármin Szeles**[1,2], Ana R. Santa-Maria[1,4], Judit P. Vigh[1,2,3], Luíza Santa Brígida de Barros Góes[1,2,5,6], Anna E. Kocsis[1,2,3], Zoltán Rakonczay[7], Péter Hegyi[8], Mária A. Deli[1], Fruzsina R. Walter[2]

[1]*Biological Barriers Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary*
[2]*HAS-HUN-REN BRC Lendület "Momentum" Translational Lab-on-a-chip models Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary*
[3]*Doctoral School of Biology, University of Szeged, Szeged, Hungary*
[4]*Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA, USA*
[5]*Instituto de Química de São Carlos, Universidade de São Paulo 400, Brazil*
[6]*Instituto Nacional de Ciência e Tecnologia de Bioanalítica Lauro Kubota – INCTBio-LK Campinas SP, Brazil,*
[7]*Department of Pathophysiology, University of Szeged, Szeged, Hungary*
[8]*Translational Pancreatology Research Group, Interdisciplinary Center of Excellence for Research Development and Innovation University of Szeged, Hungary*

Acute pancreatitis (AP) is one of the most frequent inflammatory gastrointestinal disorders which requires urgent hospitalization. Approximately 4% of AP patients experience neurological symptoms such as disturbance of consciousness during disease and among the severe cases, around 10% develop significant neurological complication resulting in pancreatic encephalopathy. Our research group previously demonstrated increased blood-brain barrier (BBB) permeability in a non-invasive rat model of AP. The present study aimed to investigate the potential damage caused by AP serum biomarkers on an in vitro model of the BBB. Serum samples were collected from patients with mild, moderate, and severe AP, as well as from healthy individuals. BBB disruption was assessed by measuring the levels of neuron-specific enolase (NSE), claudin-5, glial fibrillary acidic protein (GFAP) and S100β biomarker proteins in the blood serum of AP patients and healthy individuals. Human brain endothelial cells (hCMEC/D3 and stem cell derived) either in mono- or co-culture were treated with 20% human serum to evaluate the functional effects. Assays included permeability testing, transendothelial electrical resistance (TEER), P-glycoprotein activity assay and measurements of the release of reactive oxygen species (ROS) and nitric oxide (NO) production. For the morphological analysis PECAM, ICAM, VCAM, Claudin-5, Occludin, β-catenin and ZO-1 were stained. Our findings revealed elevated serum levels of NSE, GFAP, S100β and Claudin-5 in all AP severity groups, indicating decreased BBB integrity. Serum treatment reduced electrical resistance, increased permeability, and altered interendothelial junctional morphology in human brain endothelial cells. Moreover, ROS and NO production were also significantly elevated. These results indicate that sera from AP patients affect key BBB characteristics, including barrier integrity, oxidative stress levels, and junctional structure. The presence of BBB leakage markers in patients across all AP severity categories highlights the neurological risks associated with the disease and underscores the need for further investigation.

Acknowledgement: The project is supported by the Hungarian National Research Development and Innovation Office of Hungary (K143766, 2024-1.2.2-ERA_NET-2024-00018 and OTKA PD-128480; ADV153360 for F.R.W.). F.R.W. is also funded by the Lendület "Momentum" Research Grant (LP2025-22/2025).

Poster 12

The effects of paracellular barrier tightening on the structure and functions of focal adhesions in a human blood-brain barrier model

Lucien Lemaitre[1*], Ilona Gróf[1*], Gergő Porkoláb[1,2*], Imola Rajmón[1,3], Róbert Horváth[1,3], Mária A. Deli[1]

[1]*Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary*
[2]*Smurfit Institute of Genetics, Trinity College Dublin, D02 VF25 Dublin, Ireland*
[3]*Institute of Technical Physics and Materials Science, HUN-REN Centre for Energy Research, Budapest, Hungary*
**Authors contributed equally*

Our research group has identified a small molecular combination (cARLA) increasing blood-brain barrier (BBB) properties in human hematopoietic stem cell-differentiated endothelial cells via the increased expression and membrane localization of BBB dominant tight junction protein claudin-5. In cARLA treated endothelial cells we also observed perinuclear actin cytoskeletal structures, which we hypothesized to be focal adhesions. Our aim was to investigate the effects of cARLA on the structural morphology and functions of

brain endothelial focal adhesions.

Focal adhesion proteins: vinculin, zyxin and p-FAK were immunostained, the actin cytoskeleton and the nuclei was fluorescently labeled, and studied by a Leica Stellaris confocal microscope. Changes in cell adhesion force were studied by FluidFM, and adhesion kinetics by a label-free resonant waveguide grating biosensor.

The adhesion kinetics of endothelial cells was changed by cARLA, an early increase (0-2 h), followed by a decrease at the 24 and 48 hour time-points were measured by the optical biosensor. The structure of focal adhesions were reorganized after cARLA treatment. The number of cells with diffuse focal adhesions decreased, while the number of cells with perinuclear adhesions and perinuclear actin increased at 24 and 48 hours. While the overall adhesion energy and the cell-cell adhesion forces were increased, the cell-extracellular matrix

adhesion forces, reflecting focal adhesion functionality, did not change as measured by FluidFM.

We described for the first time how tightening of the paracellular barrier affects focal adhesion structure and function in human brain endothelial cells, contributing to a better understanding of cell mechanobiology at the BBB.

Poster 13

Astrocyte-activated NLRP3 inflammasome promotes brain metastasis formation

Adél Lüvi[1], Ádám Mészáros[1], Kinga Molnár[1], Csilla Fazakas[1], Tamás Dudás[1], Attila E. Farkas[1], István A. Krizbai[1], Imola Wilhelm[1]

[1]*Biological Research Centre, Szeged, Hungary*

Introduction:

Triple negative breast cancer-derived brain metastases are one of the most common types, having a very poor prognosis. The inflammatory processes occurring in the brain metastatic microenvironment may worsen the course of the disease, facilitating the growth and development of metastases. One of the most potent cytokines responsible for these inflammatory processes is IL-1β, primarily produced in an inflammasome-dependent manner.

Aims of the Research:

Here we aimed to understand role of the NLRP3 inflammasome in the development of brain metastases. Moreover, we wanted to determine the cell type potentially responsible for inflammasome activation.

Introducing the applied methods:

In our experiments, we used an *in vivo* brain metastasis model. We injected triple negative breast cancer cells in the right common carotid artery of the mice and examined the metastatic lesions after 2, 5 and 7 days using immunofluorescence staining and western blot.

Achieved results:

By immunofluorescence staining, we detected NLRP3 and ASC inflammasome components and the IL-1β cytokine specifically expressed only in peritumoral astrocytes. We observed that the extent of IL-1β expression was dependent on the time spent from the inoculation of the tumor cells and also on the size of the tumor lesion. By administering MCC950, a specific NLRP3 inflammasome inhibitor, significantly fewer and smaller tumors developed in the brains of the animals.

Conclusions:

We conclude that the active IL-1β cytokine produced by NLRP3 inflammasomes in peritumoral astrocytes promotes tumor growth in the brain. Specific inhibition of inflammasomes decreases the number and size of tumors developing in the brain. Our findings suggest that targeting inflammasomes could be an effective therapeutic strategy in controlling brain metastases.

Poster 14

Microplastics and the blood-brain barrier

Petra Majerova[1], Andrej Kovac[1]

[1]*Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia*

Microparticles are particles with a diameter ranging from 1 to 1000 micrometers. Microparticles are found in our environment, including drinking water, food, and air, making human exposure inevitable. The blood-brain barrier (BBB) is a highly selective barrier that protects the central nervous system (CNS) by preventing most substances in the blood from entering brain tissue. Understanding how microplastics interact with the BBB has become a critical area of research to determine the potential neurological risks associated with microplastic exposure. Although the precise mechanisms by which microplastics cross the BBB remain incompletely characterized, several pathways have been proposed, including passive diffusion, endocytosis, and transcytosis. To investigate the effect of microplastics on CNS barriers, we used a primary in vitro cell models of the blood–brain barrier (BBB) and blood-cerebrospinal fluid (BCSFB). Alterations in endothelial protein expression were analyzed. To characterize changes in protein expression we used mass-spectrometry. Aliquots of purified peptide mixtures (500 ng) were separated using an Acquity M-Class UHPLC system (Waters, Milford, MA, USA). Peptides were introduced into a nanoEase HSS T3 C18 analytical column and analyzed on a ZenoTOF 7600+ time-of-flight mass spectrometer (Sciex, USA). Spectra were acquired in a data-independent acquisition mode (SWATH), and data processing was performed using PEAKS Studio 13 software.

Acknowledgement: The project is supported by the Interreg SK-AT (MIND), APVV-22-0313, VEGA 2/0075/24.

Poster 15

Clazosentan suppresses endothelin-1-induced ROS production and metabolic alterations in cerebral capillary pericytes

Hiroki Nagatsuka[1], Genki Chikamatsu[3], Eri Shiozaki[2,3], Yoichi Morofuji[1]

[1]*Department of Neurosurgery, Showa Medical University, Tokyo, Japan*
[2]*Department of Neurosurgery, Sasebo City General Hospital, Sasebo, Japan*
[3]*Department of Neurosurgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan*

Background and Purpose: Cerebral vasospasm and delayed cerebral ischemia following subarachnoid hemorrhage (SAH) significantly worsen patient outcomes. Endothelin-1 (ET-1), a potent vasoconstrictor peptide, is implicated in this pathophysiology. Clazosentan sodium, a selective endothelin-A receptor (ETAR) antagonist, was approved in Japan in 2022, marking a significant therapeutic advancement. However, its in vitro effects on individual cell types and intercellular interactions within the blood-brain barrier (BBB) remain poorly understood. Pericytes, which form the BBB alongside capillary endothelial cells and astrocytes, regulate microcirculation in brain capillaries where vascular smooth muscle is absent. This study evaluated the effects of ET-1 and clazosentan on BBB function and pericyte metabolism using rat-derived primary cell cultures.

Methods: We isolated brain capillary endothelial cells and pericytes from 3-week-old Wistar rats. An in vitro BBB model was constructed using co-culture systems on semi-permeable membranes (0.4 μm pore size). BBB integrity was assessed by transendothelial electrical resistance (TEER) measurements. Pericyte contractility was evaluated using real-time impedance monitoring. Cellular metabolism was analyzed using a Seahorse XF Analyzer, measuring oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Reactive oxygen species (ROS) production was quantified using DCFH-DA fluorescence assays. Western blot analysis assessed ETAR protein expression.

Results: ET-1 administration did not affect TEER in endothelial-pericyte co-cultures, suggesting minimal direct impact on tight junction function. However, ET-1 dose-dependently induced intracellular ROS production in pericytes but not in endothelial cells. ET-1 significantly enhanced both mitochondrial respiration (OCR) and glycolysis (ECAR) in pericytes via ETAR activation. Pretreatment with clazosentan suppressed ET-1-induced ROS generation, metabolic alterations, and pericyte contraction. Furthermore, clazosentan inhibited ET-1-induced ETAR protein upregulation, thereby preventing excessive pericyte contraction responses.

Conclusions: Clazosentan exerts dual protective effects on pericytes by suppressing ET-1-induced acute contraction and preventing subsequent ETAR upregulation-mediated excessive contractility. These findings suggest that ET-1 modulation may protect pericytes from oxidative stress-induced injury, potentially offering novel therapeutic strategies for microcirculatory dysfunction not only in SAH but also in other cerebrovascular and neurodegenerative diseases where pericyte dysfunction contributes to pathology.

Poster 16

Modulation and measurement of endothelial and epithelial barrier integrity by FluidFM

Imola Rajmon[1,2,3], Inna Székács[1], Mária A. Deli[2], Róbert Horváth[1,2]

[1]*Nanobiosensorics Laboratory, Centre for Energy Research, Institute of Technical Physics and Materials Science, HUN-REN, 1121 Budapest, Hungary*
[2]*Institute of Biophysics, Biological Research Centre, Hungarian Research Network, Szeged H-6726, Hungary*
[3]*Doctoral School of Biology, University of Szeged, Szeged H-6720, Hungary*

With today's single-cell techniques, we can explore tissues at single cell level, deepening our understanding of cellular heterogeneity and identifying potential subpopulations. This knowledge can lead to novel health and medical applications. Notable techniques include fluidic force microscopy (FluidFM). FluidFM uses a hollow, microfabricated cantilever connected to a liquid reservoir and pressure controller, allowing precise, gentle interaction with individual cells.

One of its applications is adhesion force measurements, which use cantilevers with circular openings. By generating negative pressure in the microfluidic channel, a portion of the cell's upper membrane is drawn into the channel, creating a tight contact between the cell and the probe. Then, by lifting the probe, the cell detaches from the culture surface or another cell, and the force required for this detachment can be measured.

In another important application, a probe ending in a pyramidal tip is used to pierce the cell membrane, and under precise pressure and time control, material is injected into the cell. Using this technique, we injected fluorescent dye into human endothelial and Vero epithelial cells. In the present work we summarize the results of our preliminary experiments concerning barrier biomechanical integrity and dye transfer between neighbouring cells. The optimal injection and cell uptake parameters were determined for both cell types, followed by a comparison of their adhesion forces.

Poster 17

Protection of the blood-brain barrier in the cell culture model of ischemic stroke

Koppány Párdi[1], Anikó Szecskó[1,2], Zuhao Cui[1,2], Gergő Porkoláb[1,+], Zsófia Hoyk[1], Csilla Kovács[1], Janet Folasade Adegbite[1,++], Nárcisz M. Cser[1], László Dér[3], Krisztina Nagy[3,4], Virág Fodor[1], Mária A. Deli[1], Szilvia Veszelka[1]

[1]*Biological Barriers Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary*
[2]*Doctoral School of Biology, University of Szeged, Szeged, Hungary*
[3]*Biophotonics and Biomicrofluidics Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary*
[4]*Department of Experimental Physics, Institute of Physics, University of Szeged, Szeged, Hungary*
[+]*Present address: Smurfit Institute of Genetics, Trinity College Dublin, Ireland, FutureNeuro Research Ireland Centre, Smurfit Institute of Genetics, Trinity College Dublin*
[++]*Present address: University of Bergen, Bergen, Norway*

Stroke is the second leading cause of death and the third leading cause of long-term disability worldwide. The pathological processes triggered by oxygen-glucose deprivation in ischemic stroke damage not only neurons and glial cells, but also the blood-brain barrier (BBB). Its treatment is a clinical challenge due to the risk of reperfusion injury and limited efficacy of thrombolytic therapies, therefore, novel therapeutic approaches are needed. Histone deacetylase inhibitors (HDACi) have emerged as neuroprotective agents in stroke models, but their effect on preserving BBB integrity is still unexplored. Our aim was to investigate the effects of the HDACi suberoylanilide hydroxamic acid (SAHA) on BBB changes in a cell culture model of ischemic stroke.

In our experiments, the effects of SAHA were tested on a human BBB co-culture model under normoxia and during a 24-hour reoxygenation (OGD/R) following a 6-hour oxygen-glucose deprivation (OGD).

SAHA promoted BBB protection against OGD/R by increasing transendothelial electrical resistance and decreasing BBB permeability. SAHA also increased the level of tight junction protein claudin-5, and several ECM components associated with as glycocalyx and basement membrane. Moreover, SAHA downregulated proliferation, had a significant impact on endothelial cell morphology, and upregulated non-canonical Wnt signalling.

Our results suggest that SAHA could be a potential therapeutic drug for the treatment of ischemic stroke via BBB protection. Since SAHA has already been approved for human use as the anticancer drug vorinostat, its repurposing to restore BBB functions and prevent post-stroke damages may be greatly facilitated.

Acknowledgement: This work was funded by the National Research, Development and Innovation Fund (no.143233, FK_22). A.S. was supported by Gedeon Richter Talentum Foundation and EKÖP-517-SZTE. K.P. was supported by the Gedeon Richter Centenarium Foundation and EKÖP-415-SZTE. K.P. and V.F. were supported by the National Academy of Scientist Education Program of the National Biomedical Foundation under the sponsorship of the Hungarian Ministry of Culture and Innovation.

Poster 18

Neuroprotective roles of HSPB1 in a mouse model of Alzheimer's disease

Bettina Rákóczi[1], Zsófia Ruppert[1], Mária Péter[1], Gábor Balogh[1], Ede Migh[1], László Vígh[1], Zsolt Török[1], Melinda E. Tóth[1]

[1]*Institute of Biochemistry, HUN-REN Biological Research Centre, Szeged*

Introduction: Heat shock proteins (HSPs) are evolutionarily conserved chaperones that play an important role in maintaining cellular protein homeostasis. Therefore, they may be promising therapeutic targets for treating protein misfolding disorders, including Alzheimer's disease (AD). Indeed, we previously confirmed in a transgenic mouse model of AD, that overexpression of the small heat shock protein, HSPB1 ameliorated certain symptoms of the disease, such as amyloid plaque formation and memory impairment. Although the chaperone function of HSPB1 is essential to its neuroprotective effects, there is increasing evidence that HSPs also have several additional functions.

Methods: AD model (APP/PS1) mouse strain was crossed with an HSPB1 overexpressing line. Gene expression levels of cytokines and glia activation markers were investigated by qPCR in the hippocampus of 12-month-old animals. Moreover, we applied a spatially resolved lipidomics workflow that integrates laser microdissection microscopy with quantitative lipidomics to uncover regional differences between plaque-rich areas and non-plaque areas

Results: Due to a high incidence of seizures, female AD model mice show a higher mortality rate, which was remarkably reduced by HSPB1 overexpression. This is in line with our previous experiment, where HSPB1 overexpression was found to normalize the increased synaptic excitability in APP/PS1 mice, which may be related to the reduced seizure-susceptibility of the animals. Moreover, emerging evidence suggests a complex regulatory role for HSPs in inflammation. Here we show that HSPB1 does not further exacerbate AD-related chronic inflammation, but instead we detected a milder cytokine expression and a slight increase in markers of M2 microglial activation, suggesting a shift towards anti-inflammatory actions and tissue repair. Lipidomic analysis revealed statistically significant alterations across multiple lipid classes between the wild-type and APP/PS1 mice. Importantly, a subset of AD-associated lipid dysregulations was normalized, at least partially, by HSPB1 overexpression.

Conclusion: The above results suggest that HSPB1 not only has a chaperone function, but also plays a role in the regulation of synaptic function, inflammation, and lipid metabolism, all of which may contribute to its protective effect in AD.

Acknowledgement: This work was supported by funding from NKFIH FK138390 and TKP2021-EGA-09.

Poster 19

Traumatic brain injury induces senescence in the cells of the neurovascular unit

Tejal Shreeya[1,2], Zsófia Hernádi[1], Imola Wilhelm[1], Endre Czeiter[3], Krisztina Amrein[3], Zsolt Kristóf Bali[4], Nóra Bruszt[4], István Hernádi[4], Attila E. Farkas[1], István Krizbai[1]

[1]*Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungarian Research Network, Szeged, Hungary*
[2]*Doctoral School of Experimental and Preventive Medicine, University of Szeged, Szeged, Hungary*
[3]*Neurotrauma Research Group, Szentágothai Research Centre, University of Pécs, Hungary*
[4]*Translational Neuroscience Research Group, Szentágothai Research Centre, University of Pécs, Hungary*

Introduction:

Traumatic brain injury (TBI) is one of the leading causes of disability. It induces vascular damage, accelerates cellular senescence, impairs repair mechanisms, contributes to inflammaging, and cognitive aging. Cellular senescence caused by double-stranded DNA damage was identified as the mechanism driven by brain dysfunction after TBI. This study aims to investigate the cellular senescence in neurovascular unit cells in response to traumatic brain injury in rat brain sections.

Methods and Materials:

Marmarou weight drop model was used to induce TBI in rats. Animals were divided into 4 groups: severe TBI (STBI), mild TBI (MTBI), repetitive mild TBI (rMTBI), and control (rSHAM) at two time points: 24 hours and 4 weeks. Brain sections were co-stained with a senescence-specific marker (γH2AX) and cell type-specific markers (CD31, GFAP, Iba1, and PDGFRβ for endothelial cells, astrocytes, microglia, and pericytes) and imaged using epifluorescence and laser confocal microscopy.

Results:

A statistically significant number of astrocytes were γH2AX positive in STBI after 24 hours of injury. Microglia were γH2AX positive in statistically significant numbers in STBI and MTBI after 24 hours. Senescent astrocytes and microglia disappeared after 4 weeks. No γH2AX positive pericytes were found in any of the experimental groups. Senescent endothelial cells appeared in response to TBI only after 4 weeks, but this was only statistically significant in the cortex in STBI. Behavioural testing found no significant changes.

Conclusions:

Our data shows that TBI-induced senescence is cell type specific and its dynamics are opposite for glial cells and endothelial cells, while pericytes do not take part in this response at the time points tested.

All necessary ethical approvals have been received for the aforementioned research.

Acknowledgements: This research has also been supported by the University Research Scholarship Program (EKÖP-2024) Grant (5K 102 A202).

Poster 20

Complex temporal activity patterns replayed with fast 3D acousto-optical stimulation for partial visual restoration

Gergely Szalay[1,2*], Linda Judák[1,2*], Pál Maák[3], András Fehér[1], Andrius Plauska[1], Abhrajyoti Chakrabarti[1], Gábor Juhász[1], Balázs Tarján[1], Máté Veress[1], Zoltán Szadai[1,2], Balázs Rózsa[1,2,4]

[1]*BrainVisionCenter, Budapest-1094, Hungary*
[2]*Laboratory of 3D functional network and dendritic imaging, HUN-REN, Budapest-1083, Hungary*
[3]*Department of Atomic Physics, Budapest University of Technology and Economics, Budapest-1111, Hungary*
[4]*The Faculty of Information Technology, Pázmány Péter University, Budapest-1083, Hungary*
**Authors contributed equally*

Two-photon optogenetics enables cell-resolved mapping and patterned manipulation of neuronal circuits, supporting applications from high-throughput connectivity mapping to causal tests of population codes of perception. However, because cortical computations unfold in intrinsically three-dimensional (3D) networks, existing strategies for combined 3D readout and 3D manipulation are constrained by fundamental trade-offs between temporal and spatial resolution10 and the ability to access widely distributed targets within large volumes.

Here we introduce a dual-wavelength acousto-optic (AO) scanning system that overcomes these constraints by enabling microsecond-scale switching between imaging and photostimulation while maintaining diffraction-limited resolution and true 3D random-access targeting throughout the entire imaging volume. This unified optical framework supports rapid functional readout together with precise, distributed manipulation of neuronal networks at single-cell level without sacrificing spatiotemporal performance. Motivated by evidence that perceptual representations are embedded in structured temporal activity patterns rather than simple co-firing, we leverage this capability to replay endogenous dynamics with fine control over timing, power, and 3D targeting at single-cell resolution. Using this approach, we achieve simultaneous recording and stimulation of up to 850 neurons across a 700 µm z-range in mouse primary visual cortex and demonstrate the reactivation of complex activity sequences with up to millisecond-precision. In behaving mice, selective replay of perceptually evoked 3D activity patterns enhanced performance in visual detection and discrimination tasks, providing causal evidence that temporally structured ensemble dynamics contribute to perception. Together, our results establish a general framework for cell-resolved, temporally structured control of neural activity in three dimensions and lay groundwork for future closed-loop optical manipulation of cortical circuits.

Poster 21

The protection of the blood-brain barrier by a small-molecule cocktail, cARLA in a cell culture model of ischemic stroke

Anikó Szecskó[1,2], Koppány Párdi[1], Zuhao Cui[1,2], Gergő Porkoláb[1,3], Zsófia Hoyk[1], Csilla Kovács[1], Nárcisz M. Cser[1], Krisztina Tóth[4], Ádám Dénes[4], Csilla Sajben[5], Roland Tengölics[5], Mária A. Deli[1], Szilvia Veszelka[1]

[1]*Biological Barriers Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary*
[2]*Doctoral School of Biology, University of Szeged, Szeged, Hungary*
[3]*Smurfit Institute of Genetics, Trinity College Dublin, Ireland, FutureNeuro Research Ireland Centre, Smurfit Institute of Genetics, Trinity College Dublin*
[4]*Neuroimmunology Research Group, HUN-REN Institute of Experimental Medicine, Budapest, Hungary*
[5]*Metabolomics Laboratory, Core Facilities, HUN-REN Biological Research Centre, Szeged, Hungary*

Stroke is the second leading cause of death, and 88% of all stroke cases are ischemic triggered by a vascular occlusion caused by a thrombus. During ischemic stroke, the resulting lack of oxygen and glucose and the subsequent reperfusion leads to neuronal damage and disruption of the blood-brain barrier (BBB). Currently, there are two main approaches in clinical practice for the treatment of ischemic stroke: thrombolysis and mechanical thrombectomy. However, these procedures can only be used in larger arteries, carry significant risks and have a narrow therapeutic window (4.5 h) from symptom onset. Therefore, additional effective therapeutic approaches for treatment of ischemic stroke are urgently needed.

The small-molecule combination, cARLA, simultaneously activates cAMP and Wnt/β-catenin signaling while inhibiting the TGF-β pathway of the BBB. Previously we demonstrated that cARLA robustly enhancing BBB properties by increasing barrier tightness, glycocalyx density, and shifting gene expression toward a brain endothelial phenotype under normal conditions. Here, our aim was to investigate the effect of cARLA on restoring the function of the BBB to prevent ischemic damages.

The effects of cARLA were tested on the human model of the BBB under normoxia and during a 24-hour reoxygenation (OGD/R) following a 6-hour oxygen-glucose deprivation (OGD). The viability of brain endothelial cells decreased dramatically after OGD, whereas cARLA treatment during OGD/R significantly improved cell index and metabolic activity detected by impedance-based measurement and MTT assay. The expression of the tight junction protein claudin-5 and sialic acid residues of the glycocalyx was partially reduced after OGD, but cARLA treatment significantly increased the levels of both during reoxygenation. Moreover, cARLA influenced BBB-related gene expression, including claudin-5, and altered metabolomic profiles such as certain glycocalyx components after ischemic events.

Based on our results, cARLA may be a potential therapeutic tool for the treatment of ischemic stroke, but further research is needed to gain a deeper understanding of its mechanism of action and to develop its potential clinical applicability.

Acknowledgements: A.S. was supported by Gedeon Richter Talentum Foundation and EKÖP-517-SZTE. K.P. was supported by Gedeon Richter Centenarium Foundation and EKÖP-415-SZTE. K.P. and G.P. was supported by the National Academy of Scientist Education Program of the National Biomedical Foundation under the sponsorship of the Hungarian Ministry of Culture. S.V. was supported by the National Research Development and Innovation Office of Hungary (FK143233).

Poster 22

Increased BBB permeability to a-synuclein in dextran sulfate sodium-induced colitis mice

Fuyuko Takata[1], Junko Mizoguchi[1], Takuro Iwao[1], Yasuyoshi Tanaka[1], Akio Nakashima[2], Kazunori Sano[3], Osamu Imakyure[2], Shinya Dohgu[1]

[1]Department of Pharmaceutical Care & Health Sciences, Faculty of Pharmaceutical Sciences, Fukuoka University
[2]Department of Pharmacy, Fukuoka University Chikushi Hospital
[3]Department of Physiology and Pharmacology, Faculty of Pharmaceutical Sciences, Fukuoka University

Patients with inflammatory bowel disease (IBD) have a higher risk of developing Parkinson's disease compared with people without IBD. However, it remains unclear whether chronic bowel inflammation triggers a-synuclein accumulation in the brain which is a major cause of development of Parkinson's disease. We previously found that C57BL/6 mice treated with sodium dextran sulfate (DSS), an IBD model with colitis, showed a significant increase in brain a-synuclein, suggesting that bowel inflammation may promote a-synuclein accumulation in the brain. Since a-synuclein is a ubiquitous protein in the brain and blood, a portion of a-synuclein in the brain could be derived from blood. To evaluate whether a-synuclein in the blood is transported into brain parenchyma across the BBB under IBD conditions, DSS-treated mice were injected intravenously with ^{125}I-a-synuclein, and ^{125}I-a-synuclein levels in the brain were measured. ^{125}I-a-synuclein was transported into brain parenchyma. The blood-to-brain unidirectional influx rate (Kin) values for ^{125}I-a-synuclein in DSS-treated mice was 1.2-fold higher than that in control mice. Percent of the iv injected dose of ^{125}I-a-synuclein taken up per gram of brain tissue (%Inj/g-brain) was significantly increased compared with the control mice. Brain pericytes play a key role in maintaining the functional integrity of the BBB. However, it has not been well known whether IBD pathology affects the function of pericytes at the BBB. DSS-treated mice exhibited reduced expression of PDGFRβ, a marker of pericytes, and decreased occludin levels in brain microvessels, suggesting that pericyte function at the BBB was altered under IBD conditions. These findings raise a possibility that BBB dysfunction driven by pericyte impairment under IBD condition facilitates transport of a-synuclein from blood to brain parenchyma through the BBB, leading to the elevated a-synuclein in the brain of IBD mice.

Poster 23

Albumin-induced blood-brain barrier dysfunction and its role in neuroinflammation relevant to epileptogenesis

Daiki Uchida[1], Yoichi Morofuji[2], Daisuke Watanabe[3], Shiro Baba[1], Takayuki Matsuo[1]

[1]*Department of Neurosurgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan*
[2]*Department of Neurosurgery, Showa Medical University School of Medicine, Tokyo, Japan*
[3]*BBB Laboratory, PharmaCo-Cell Company Ltd., Nagasaki, Japan*

Background and Aims:

Acquired structural epilepsy is increasing worldwide, and no current therapy prevents epileptogenesis. Neuroinflammation triggered by blood–brain barrier (BBB) disruption is considered a key mechanism; however, the early effects of blood-derived factors on BBB integrity remain unclear. This study aimed to examine how albumin deposition affects BBB function using a physiologically relevant in vitro BBB model.

Methods:

We used a Transwell in vitro BBB model consisting of primary rat brain capillary endothelial cells, pericytes, and astrocytes. Bovine serum albumin, low-endotoxin albumin, or fatty acid–free albumin at different concentrations was applied to the luminal side, abluminal side, or both compartments to mimic early BBB disruption. BBB integrity was assessed by transendothelial electrical resistance (TEER) and sodium fluorescein (Na-F) permeability assays.

Results:

Albumin exposure induced a concentration-dependent decrease in TEER. BBB dysfunction was more prominent with abluminal exposure than with luminal exposure and progressed most rapidly under bilateral exposure. Bilateral exposure to ≥5% low-endotoxin albumin caused immediate and irreversible BBB impairment. Na-F permeability increased prominently after bilateral exposure to ≥5% low-endotoxin albumin, whereas fatty acid–free albumin did not affect permeability. These findings indicate that the BBB is more vulnerable to albumin exposure from the brain side and that barrier dysfunction accelerates under bilateral exposure. Furthermore, the results suggest that albumin-associated bioactive lipids, rather than albumin itself, contribute to BBB disruption.

Conclusions:

Albumin induces site- and concentration-dependent BBB dysfunction in an in vitro BBB model. BBB disruption mediated by albumin-associated factors may promote neuroinflammatory processes relevant to epileptogenesis, highlighting BBB protection as a potential preventive therapeutic strategy.

Poster 24

Interfacial water organization in glycocalyx model systems: towards understanding blood-brain barrier hydration structure

Dános Sebestyén Varga[1,2,3,4], Ilona Gróf[1], Lóránd Kelemen[1], Mária Anna Deli[1], Róbert Horváth[1,3], Zsuzsanna Heiner[4], András Dér[1]

[1]*Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary*
[2]*Doctoral School of Multidisciplinary Medical Sciences, University of Szeged, Szeged, Hungary*
[3]*Nanobiosensorics Laboratory, HUN-REN Centre for Energy Research, Budapest, Hungary*
[4]*Institute of Chemistry and School of Analytical Sciences Adlershof, Humboldt-Universität zu Berlin, Berlin, Germany*

The blood-brain barrier (BBB) endothelial glycocalyx (GLX) maintains barrier integrity through mechanisms incompletely understood at the molecular level. While the GLX's role in selective permeability is established, the contribution of organized interfacial water remains unexplored. We hypothesize that oriented water structures within the GLX contribute steric and electrostatic barriers complementing tight junctions.

To investigate this, we developed a systematic approach using vibrational sum-frequency generation (VSFG) spectroscopy – a surface-selective technique probing oriented molecules at interfaces – combined with attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy to study progressively complex GLX-relevant model systems.

We examined lipid monolayers at the air-water interface with GLX-relevant phospholipids and glycosphingolipids. VSFG measurements revealed distinct, oriented water signatures across regions, with spectral features varying with headgroup chemistry and surface pressure. Complementary studies on aqueous solutions of glycosaminoglycans (hyaluronic acid, chondroitin sulfate) showed concentration-dependent water orientation at the air-solution interface. Additionally, ATR-FTIR analysis quantified hydrogen bonding networks and identified specific carbohydrate-water interactions through characteristic spectral shifts.

These model system studies establish baseline interfacial water signatures for key GLX components. Our current work is progressing toward native membrane fragments to bridge the complexity gap between simplified models and the intact BBB endothelium. This systematic approach provides molecular-level insights into how GLX components organize interfacial water, potentially contributing to barrier function. Understanding these fundamental hydration structures may inform BBB pathophysiology in neurological disorders where GLX degradation occurs.

Poster 25

Effects of clinically used iodinated contrast agents on blood-brain barrier integrity after oxygen-glucose deprivation

Judit P. Vigh[1,2;*], Anna E. Kocsis[1,2,*], Ilona Gróf[1,*], Ana Raquel Santa-Maria[1,3], Yuki Matsunaga[4], Daisuke Watanabe[5], Yoichi Morofuji[6], Mária A. Deli[1,*], Fruzsina R. Walter[1,*,#]

[1]*Biological Barriers Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary*
[2]*Doctoral School of Biology, University of Szeged, Szeged, Hungary*
[3]*Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA, USA*
[4]*Department of Neurosurgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan*
[5]*BBB Laboratory, PharmaCo-Cell Company, Ltd., Nagasaki, Japan*
[6]*Showa Medical University, School of Medicine, Tokyo, Japan*
**Authors contributed equally*
[#]*Corresponding author: walter.fruzsina@brc.hu*

Contrast media (CM) are used for imaging cerebrovascular diseases, like stroke. During neurointerventions extravasation of CM to brain parenchyma leads to serious side effects. The aim of this study was to investigate the effect of CM on the integrity of blood-brain barrier (BBB) models in conditions mimicking physiology and stroke. We used a BBB co-culture model based on rat primary brain endothelial cells, astrocytes and pericytes. To model stroke, oxygen glucose deprivation (OGD) was performed. Two types of CM were examined, iodixanol, an iso-osmolar, and iopamidol, a hyperosmolar agent. CM treatments lasted for 30 minutes followed by 24-hour recovery. Barrier integrity was measured by real-time impedance analysis of brain endothelial monolayers, and transendothelial electrical resistance and permeability on the co-culture BBB model. Tight junction morphology was evaluated by immunostaining. Short term direct CM exposure after OGD caused a significant drop in the impedance of the brain endothelial monolayers without full recovery. The barrier integrity also decreased at 1% and 10% CM concentrations, but these groups recovered to the level of the control group after 24 hours. In normoxia CM disturbed barrier integrity of the co-culture model measured by junctional morphology, resistance and permeability assays, which was more pronounced after OGD. The damaging effect of hyperosmolar CM was higher on the integrity of the BBB both in normoxia and OGD than that of the iso-osmotic CM. Our data support the clinical observations on the negative impact of hyperosmolar CM used in neurointerventional and endovascular therapy, especially in stroke patients.

Acknowledgement: Japanese-Hungarian bilateral grants between the two collaborator laboratories are secured by the Hungarian Academy of Sciences and the Japan Society for the Promotion of Science. The project is also supported by the Hungarian National Research Development and Innovation Office of Hungary (K143766, 2024-1.2.2-ERA_NET-2024-00018 and ADV153360 for F.R.W.). F.R.W. is also funded by the Lendület "Momentum" Research Grant (LP2025-22/2025).

Poster 26

Development of a human in vitro blood–brain barrier model for pharmacoresistant epilepsy

Emílie Kučerová[1], **Jitka Viktorová[1]**

[1]University of Chemistry and Technology Prague, Technická 5, 166 28, Prague 6, Czechia

During neurological disorders such as pharmacoresistant epilepsy, pathological alterations of the blood–brain barrier (BBB), including increased efflux transporter expression and enhanced drug-metabolizing activity, can severely limit drug delivery to the brain and contribute to treatment failure[1, 2]. While advanced *in vitro* BBB models have improved physiological relevance, models that reflect disease-specific and pharmacoresistant states remain limited[3].

Here, we present our progress toward the development of a human *in vitro* BBB model that recapitulates pharmacoresistance observed during chronic epilepsy treatment. The model is established in a transwell system using human cerebral endothelial cells (HBEC-5i), astrocytes, and vascular pericytes. Optimization parameters include cell arrangement, insert format, membrane coating, and cultivation time. Barrier integrity is assessed by transendothelial electrical resistance (TEER) and fluorescein permeability measurements. To induce pharmacoresistance, HBEC-5i cells are exposed to sub-toxic concentrations of selected antiepileptic drugs over prolonged cultivation periods, mimicking adaptive responses observed during chronic treatment. Resistance development is evaluated through changes in drug toxicity and transcriptomic profiles.

Our findings identify several key factors supporting BBB formation, including the use of larger (12well) transwell inserts with polytetrafluoroethylene membranes coated with collagen, fibronectin, and poly-L-lysine. The highest barrier integrity is achieved in a contact co-culture configuration, with endothelial cells seeded apically and astrocytes and pericytes co-cultured basolaterally. Importantly, exposure to valproate induces pharmacoresistant features within four weeks.

Together, these findings support the development of a more predictive *in vitro* platform for antiepileptic drug screening and the study of BBB-mediated pharmacoresistance.

Acknowledgements: This project is supported by the Technology Agency of the Czech Republic, No. TN02000109.

References

1. Löscher W., Potschka H.: Nat Rev Neurosci, *6*, 591 (2005).
2. Ghosh C., Gonzalez-Martinez J., Hossain M., Cucullo L., Fazio V., Janigro D., Marchi N.: Epilepsia, *51*, 1408 (2010).
3. Chaulagain, B., Gothwal, A., Lamptey, R. N. L., Trivedi, R., Mahanta, A. K., Layek, B., Singh, J.: Int J Mol Sci., *3*, 24 (2023).

Poster 27

Protective effects of isonicotinamides on BBB integrity

Katja Vuković[1], Valentina Bušić[2], Dajana Gašo Sokač[2], Maja Katalinić[1], Antonio Zandona[1]

[1]Division of Toxicology, Institute for Medical Research and Occupational Health, Ksaverska cesta 2, HR-10001 Zagreb, Croatia
[2]Faculty of Food Technology Osijek, Josip Juraj Strossmayer University of Osijek, Kuhačeva 20, HR-31000 Osijek, Croatia

The blood-brain barrier (BBB) plays a key role in maintaining homeostasis of the central nervous system, but its integrity can be impaired due to oxidative stress, which is a hallmark of the pathology of numerous neurodegenerative diseases. Previous research has shown that nicotinamide derivatives have a protective effect on the brain endothelial cells. In this research, we tested the potential of 5 selected derivatives of isonicotinamides, structurally related to nicotinamides, in protecting the immortalized human brain endothelial cell line (HBEC-5i) from oxidative stress induced by *tert*-butyl hydroperoxide (tBHP). The following assays showed that, when used as a pretreatment prior to oxidative stress exposure, isonicotinamides led to a decrease in intracellular ROS, decrease of GSH enzyme consumption, increase in superoxide dismutase activity, increase in mitochondrial membrane potential, also keeping transendothelial electrical resistance high by preserving cell barrier integrity while maintaining barrier permeability. These findings support the further studies describing the potential of isonicotinamide derivatives as protective agents against oxidative stress-induced BBB dysfunction.

Acknowledgement: This work was funded by the European Union – Next Generation EU (BioMolTox, Class: 643-02/23-01/00016, Reg. No. 533-03-23-0006), and supported by the European Regional Development Fund (project RecIMI, KK.01.1.1.02.0007).

Poster 28

Species- and model-dependent efficacy of barrier-protective compounds under ROS-induced stress

Antonio Zandona[1], Anikó Szecskó[2], Valentina Bušić[3], Dajana Gašo Sokač[3], Maja Katalinić[1], Mária Deli[2], Szilvia Veszelka[2]

[1]Division of Toxicology, Institute for Medical Research and Occupational Health, Ksaverska cesta 2, HR 10001 Zagreb, Croatia
[2]Institute of Biophysics, Biological Research Centre, Temesvári krt. 62, Szeged 6726, Hungary
[3]Faculty of Food Technology Osijek, Josip Juraj Strossmayer University of Osijek, Kuhačeva 20, HR-31000 Osijek, Croatia

In vitro blood–brain barrier (BBB) models are widely used to study oxidative stress–induced dysfunction and protective strategies; however, the choice of species and cellular origin critically influences experimental outcomes. In this study, we compared the magnitude and characteristics of oxidative stress responses and protective interventions in human pluripotent stem cell–derived brain endothelial cells (hEC) and primary rat brain endothelial cells (RBEC). Special emphasis was placed on barrier-protective nicotinamide derivatives designed to counteract reactive oxygen species (ROS)–induced BBB damage. Although both models exhibited oxidative stress–induced barrier impairment, the strength of the effects and the efficacy of the compounds differed markedly between human and rat cells. hEC generally showed stronger barrier recovery and antioxidant responses, whereas RBEC required higher concentrations to achieve comparable effects. These differences were associated with distinct cellular responses affecting redox balance, barrier integrity, and mitochondrial function, suggesting divergent underlying mechanisms. In addition, differences in culture conditions, differentiation status, and medium composition likely contributed to the observed responses. Overall, the barrier-protective efficacy of the same nicotinamide derivative depended strongly on species and cellular origin, demonstrating that BBB model selection critically shapes the interpretation of ROS-related protection.

Acknowledgements: This study was funded by the European Regional Development Fund project KK.01.1.1.02.0007, the European Union – Next Generation EU (Program Contract of 8 December 2023, Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006) and by National Research, Development and Innovation Office of Hungary (FK 143233).

Poster 29

Integrated proteomic analysis of resected tumor tissues and longitudinally collected plasma from patients with glioblastoma

Xiuyuan Zhan[1], Adam McGlinchey[2], Angela Garcia-Gallardo[2], Jeffrey O'Callaghan[2], Yosuke Hashimoto[1], Yasuo Uchida[1], Kieron Sweeney[3], Doncha O'Brien[3], Matthew Campbell[2]

[1]Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan
[2]Smurfit Institute of Genetics, Trinity College Dublin, Dublin, Ireland
[3]Department of Neurosurgery, Beaumont Hospital, Dublin, Ireland

Background:

Glioblastoma multiforme (GBM) is the most aggressive brain tumor with poor prognosis, characterized by heterogeneously disrupted blood-brain barrier (BBB). The most commonly used regimen in the treatment of GBM includes surgical resection, radiotherapy and temozolomide chemotherapy; all of these factors further cause changes in the BBB integrity and plasma profiles, yet the functional interactions between them are poorly understood. The aim of this study is to monitor changes in plasma proteome through the GBM treatment and check whether there is a correlation between plasma and tumor tissues proteome.

Materials and Methods:

Plasma was collected and dynamic contrast-enhanced (DCE)-MRI images were acquired from 39 GBM patients at pre-surgery, intra-surgery, 72 h post-surgery, 1month post-surgery, 1month post-chemotherapy in Beaumont Hospital with informed consent. Surgically resected tumor tissues were broken down into 3 groups based on DCE-MRI: "enhancing" (where gadobenate dimeglumine was highly permeable), necrotic core, and peritumoral "non-enhancing" regions. Quantitative proteomics was carried out using a nano-LC-MS/MS system consisting of Vanquish Neo UHPLC and ZenoTOF7600, followed by DIA-NN analysis in Hiroshima University.

Results:

Plasma proteomic analysis revealed phase-specific dynamics: acute postoperative complement activation (MASP2, SERPINA5) and lipid suppression (APOE, APOA2); subacute ECM (extracellular matrix) remodeling (TGFBI, EFEMP1) with sustained complement recruitment (C1QA); and therapy-associated angiogenesis and stress markers (HGFAC, NPM2). Proteins that are clearly derived from brain cells such as myelin components were also detected in some plasma samples. Functional enrichment analysis of tumor proteome identified region-specific signatures; proteins involving vesicle trafficking were highly enriched in enhancing regions, while proteins associated with synaptic signaling (GABRB1, GRM1, CACNA1A) were highly enriched in peritumoral non-enhancing regions. We are still integrating these proteomic data with clinical information such as the integrity of the BBB and the survivability.

ACKNOWLEDGEMENTS

Hungarian Neurocience Society

PharmaCo-Cell Ltd.

HUN-REN Biological Research Centre Szeged – Institute of Biophysics

Data Protection and Consent (GDPR)

Participants agree that their full name, e-mail address, title, and affiliation may appear in the conference program together with the short summary (abstract) submitted. Participants further acknowledge and consent that photographs and/or audio recordings may be taken during the conference and may be published on the conference website.
All personal data will be processed in accordance with the BRC's GDPR regulations, which are available at: www.brc.hu.

HUN-REN Biological Research Centre Szeged
2026