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Research Partners

- Laboratory for High Energy Physics (LHEP), University of Bern, Bern, Switzerland
- Institute for Research in Biomedicine, Bellinzona, Switzerland
- Centre de Physiopathologie de Toulouse-Purpan, INSERM UMR1043, Toulouse, France
- Karolinska Institutet, Huddinge, Sweden
- Centre de Recherche, Laboratoire de la barrière hémato-encéphalique, Université d'Artois, Lens, France
- Department of Neuropathology, University of Freiburg, Freiburg im Breisgau, Germany
- Department of Biomedical Engineering, University of Rochester Medical Center, NY, USA
- KU Leuven, Leuven, Belgium
- Department of Dermatology, University Hospital Zurich, Zurich, Switzerland

Research Profile

Founding of the TKI in 1950 was made possible by a donation of the Bernese Nobel laureate Theodor Kocher in 1912. Current research at the TKI is to a large part dedicated to investigate cellular and molecular mechanisms involved in neuroinflammation as they occur in multiple sclerosis or in stroke. A special focus hereby lies on studying immune cell migration into the central nervous system (CNS) during immune surveillance and neuroinflammation employing cutting-edge 3D in vitro and in vivo live cell imaging methodologies and targeted transgenic mouse models. Research competence at the TKI allows for the coordination of the Microscopy Imaging Center (MIC, www.mic.unibe.ch) and the heading of the Mouse Cryoconservation and Mouse Transgenic and Genetic Engineering Facility, a member of the transgenesis platform of the Swiss Animal Facilities Network (SAFN).

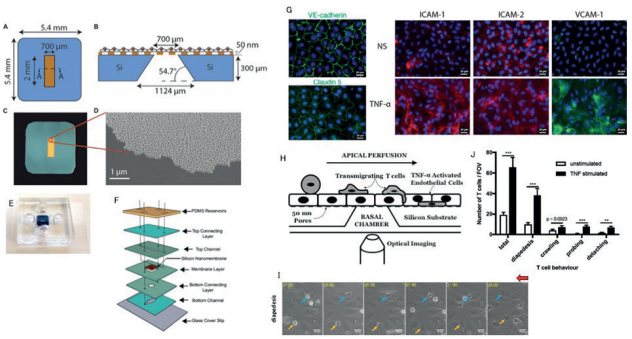
Teaching Profile

The TKI is involved in local, national and international teaching efforts providing a large portfolio of lectures and practical classes in immunology, microscopy, vascular cell biology, transgenic mouse technologies, inflammation and high end in vitro and in vivo live cell imaging for bachelor, master and graduate students in the Medical, Science and Vetsuisse Faculties. The TKI hosts the two interfaculty Graduate Schools (Graduate School for Cellular and Biomedical Sciences (www.gcb.unibe.ch) & Graduate School for Health Sciences (www.ghs.unibe.ch). In addition, coordination of the Swissuniversities supported PhD programs "Cell Migration" and "Cutting Edge Microscopy" are localized at the TKI. Britta Engelhardt is coordinator of the Horizon2020 funded international PhD student training program BtRAIN (http://www.btrain-2020.eu).

Highlights 2018

Development of a breakthrough microfluidic human in vitro cerebrovascular barrier (CVB) model

In collaboration with Dr. McGrath (University of Rochester, NY, USA), Dr. Gosselet (Université d'Artois, Lens, France) and Dr. Sallusto (IRB, Bellinzona) we have developed a microfluidic human in vitro cerebrovascular barrier (CVB) model featuring stem cell derived brain like endothelial cells (BLECs) and nanoporous silicon nitride (NPN) membranes (µSiM-CVB). The nanoscale thinness of NPN membranes combined with their high permeability and optical transparency make them an ideal scaffold for the assembly of a two-chamber in vitro microfluidic model of the blood-brain barrier (BBB). With the benefit of physiological flow and superior imaging quality, the µSiM-CVB allows to image each phase of the multi-step T-cell migration across the BBB by live cell imaging. The small scale of the µSiM-CVB allows for in vitro investigations of rare patient derived immune cells with the human BBB. We expect the µSiM-CVB to become a valuable new tool for the study of cerebrovascular pathologies ranging from neuroinflammation to metastatic cancer.

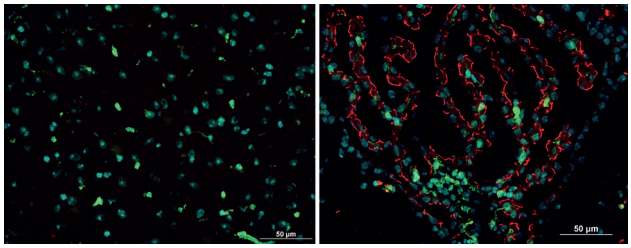


µSiM-CVB: (A) Schematic of the NPN membrane. (B) The cross-sectional schematic of the membrane. (C) Optical micrograph of the chip. (D) Scanning electron micrograph of the NPN membrane. (E,F) Flow chamber configuration. (G) Immunofluorescence staining of BLECs. (H) Schematic of the flow settings. (I) Snapshots illustrating the multi-step interaction of T cells with BLECs. (J) Analysis of T-cell interactions with BLECs.

Claudin-3 is not expressed at the blood-brain barrier

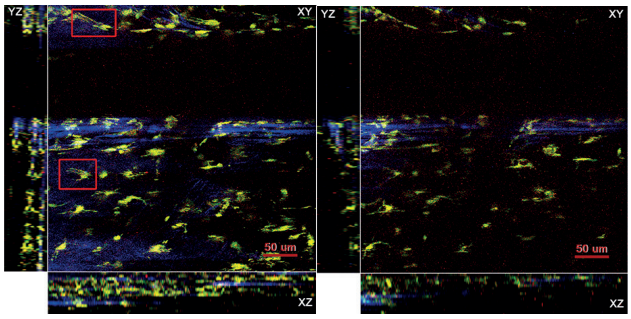
The tight junction protein claudin-3 has been proposed to play a central role in regulating integrity of brain barriers' tight junctions. By establishing and analysing claudin-3$^{-/-}$ C57BL/6J mice using bulk and single cell RNA sequencing

and direct comparative qRT-PCR analysis, we found that brain endothelial cells do not express claudin-3 mRNA. Detection of claudin-3 protein at the BBB in vivo and in vitro is rather due to junctional reactivity of anti-claudin-3 antibodies to an unknown antigen still detected in claudin-3$^{-/-}$ brain endothelium. At the same time our study did confirm expression and junctional localization of claudin-3 at the epithelial blood-cerebrospinal fluid barrier (BCSFB) of the choroid plexus. Absence of claudin-3 did however not impair brain barriers function during health and neuroinflammation in C57BL/6J mice (Dias, Coisne et al., Sci Reports, 2019, 9(1):203).



Claudin-3 is localized to tight junctions of the BCSFB. Immunofluorescent staining of frozen brain sections from WT C57BL/6, showing absence and presence of claudin-3 from the BBB (left) and the BCSFB (right), respectively. Red = claudin-3; green = endothelial cells, blue = nuclear staining. Scale bar= 50µm.

Employing real-time data processing for movement correction and cell tracking during image acquisition
Funding Unibern ID grant allowed for a collaboration of the Engelhardt group with Dr. Ariga from High Energy Physics of the University of Bern. Making use of his expertise in real-time data processing in microscopy employing parallel computing with GPUs, we established a powerful novel controllable synchronization tool allowing to use twophoton-intravital microscopy (2P-IVM) to image the cervical spinal cord, an area subjected to periodic movement due to animal breathing. The novel tool implements distortion correction in acquired images, thus enabling precise measurement of cellular motility parameters. The technology sets the stage for studying the cellular pathway of T-cell diapedesis across the BBB in vivo by 2P-IVM.



Example of the cervical spinal cord leptomeninges imaging in CX3CR1-GFP knock-in mice. Left: without synchronization tissue movement causes distortion in the acquired 3D volume. Fine structures appear as duplicates in XY projection (highlighted), and structural information in depth is lost (XZ, YZ projections). Right: with synchronization the fine cellular protrusions and the structural information is restored. Myeloid-cells (green) and collagen (blue, second harmonic generation).

Chemokines and integrins independently tune actin flow and substrate friction during intranodal migration of T cells
Although much is known about the physiological framework of T cell motility, and numerous rate-limiting molecules have been identified through loss-of-function approaches, an integrated functional concept of T cell motility is lacking. The Stein group used in vivo precision morphometry together with analysis of cytoskeletal dynamics in vitro to deconstruct the basic mechanisms of T-cell migration within lymphatic organs. In collaboration with Dr. Sixt (Vienna), we showed that the contributions of the integrin LFA-1 and the chemokine receptor CCR7 are complementary rather than positioned in a linear pathway, as they are during leukocyte extravasation from the blood vasculature. Our data demonstrate that CCR7 controls cortical actin flows, whereas integrins mediate substrate friction that is sufficient to drive locomotion in the absence of considerable surface adhesions and plasma membrane flux.

VLA-4 mediated adhesion of melanoma cells on the blood–brain barrier is a critical cue in brain metastasis formation
Melanoma is the most aggressive skin cancer in humans. One severe complication is the formation of brain metastasis. Employing live cell imaging of melanoma cells during their interactions with a mouse BBB model in vitro, we revealed a critical role of the integrin VLA-4 on melanoma cells for shear resistant arrest on and intercalation into the BBB. A tissue microarray revealed that indeed a majority of 92% of all human melanoma brain metastases stained VLA-4 positive. In conclusion, we propose VLA-4 as a target for the inhibition of brain metastasis formation in the context of personalized medicine.

Selected Competitive Grants

- Swiss National Science Foundation (31003A_170131, CRSII3_154483, 31003A_172994, 16CRSII5_170969).
- European Union: (FP7 MCA-ITN 607962nEUROinflammation; H2020-MSCA-ITN-2015 675619)
- Fidelity International Foundation, Swiss Multiple Sclerosis Society, Theodore Ott Fund, Bangerter-Rhyner Foundation, Foundation for Clinical-Experimental Cancer Research

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