(1) Submission ID#1527394

Development of a multicellular in vitro model of the meningeal blood-CSF barrier to study Neisseria meningitidis infection

Author(s)

Leo M. Endres, n/a

PhD student

Institute for Hygiene and Microbiology, University of Würzburg, Würzburg, Germany

Marvin Jungblut Jungblut, n/a

PhD student

Department of Biotechnology and Biophysics, Biocenter, University of Würzburg, Würzburg, Germany

Mustafa Divyapicigil, n/a

PhD student

Department of Biological Sciences, University of Alabama, Tuscaloosa AL, USA

Markus Sauer, n/a

Professor

Department of Biotechnology and Biophysics, Biocenter, University of Würzburg, Würzburg, Germany

Christian Stigloher, n/a

Professor

Imaging Core Facility, Biocenter, Julius-Maximilian University Würzburg, Würzburg, Germany

Myron Christodoulides, n/a

Professor

Molecular Microbiology, School of Clinical and Experimental Sciences, University of Southampton Faculty of Medicine, Southampton, UK

Brandon J. Kim, n/a

Ass. Professor

Department of Biological Sciences, University of Alabama, Tuscaloosa AL, USA

Alexandra Schubert-Unkmeir, n/a

Prof., PI

Institute for Hygiene and Microbiology, University of Würzburg, Würzburg, Germany

Background

Neisseria meningitidis (Nm, meningococcus) is a human-specific bacterium that can gain access to the central nervous system by crossing the meningeal blood-cerebrospinal fluid barrier (mBCSFB), and cause meningitis. Previous research on this host-pathogen interaction has mostly been done on immortalized brain endothelial cells (BECs) alone, which do not retain physiologically relevant barrier properties such as high transendothelial electrical resistance (TEER), and little is known about bacterial interaction with other cell types of the mBCSFB such as leptomeningeal cells (LMC). Here, we report on the development of a physiologically relevant in vitro model of the human mBCSFB using BECs derived from induced pluripotent stem cells (iPSC) or hCMEC/D3 cells in co-culture with LMCs derived from tumor biopsies to examine Nm interaction.

Aim/Methods

Barrier integrity was evaluated via TEER and sodium fluorescein (NaF) permeability. Transmission electron microscopy, confocal and super-resolution microscopy techniques were applied for model characterization and detection of interacting bacteria. Gentamicin protection and transmigration assays were conducted to determine levels of Nm adherence, invasion, and barrier transmigration. Transcriptional changes in host gene expression in response to infection were quantified using qPCR.

Results

iPSC derived ECs co-cultured with LMCs showed characteristic expression of BEC markers including tight junction proteins and reached higher TEER along with lower NaF permeability compared to monoculture. TEER remained at significantly higher levels for more than seven days. Upon infection, we detected considerable amounts of meningococci adhering to the BEC layer, whereas the number of recovered intracellular bacteria was low. Interestingly, we discovered bacteria already traversing in small numbers 6 h post-challenge, when barrier integrity was still high, suggesting a transcellular route. By 30 h, deterioration of the barrier properties has been detected, including loss of TEER and reduced expression of cell-junction components.

Conclusions

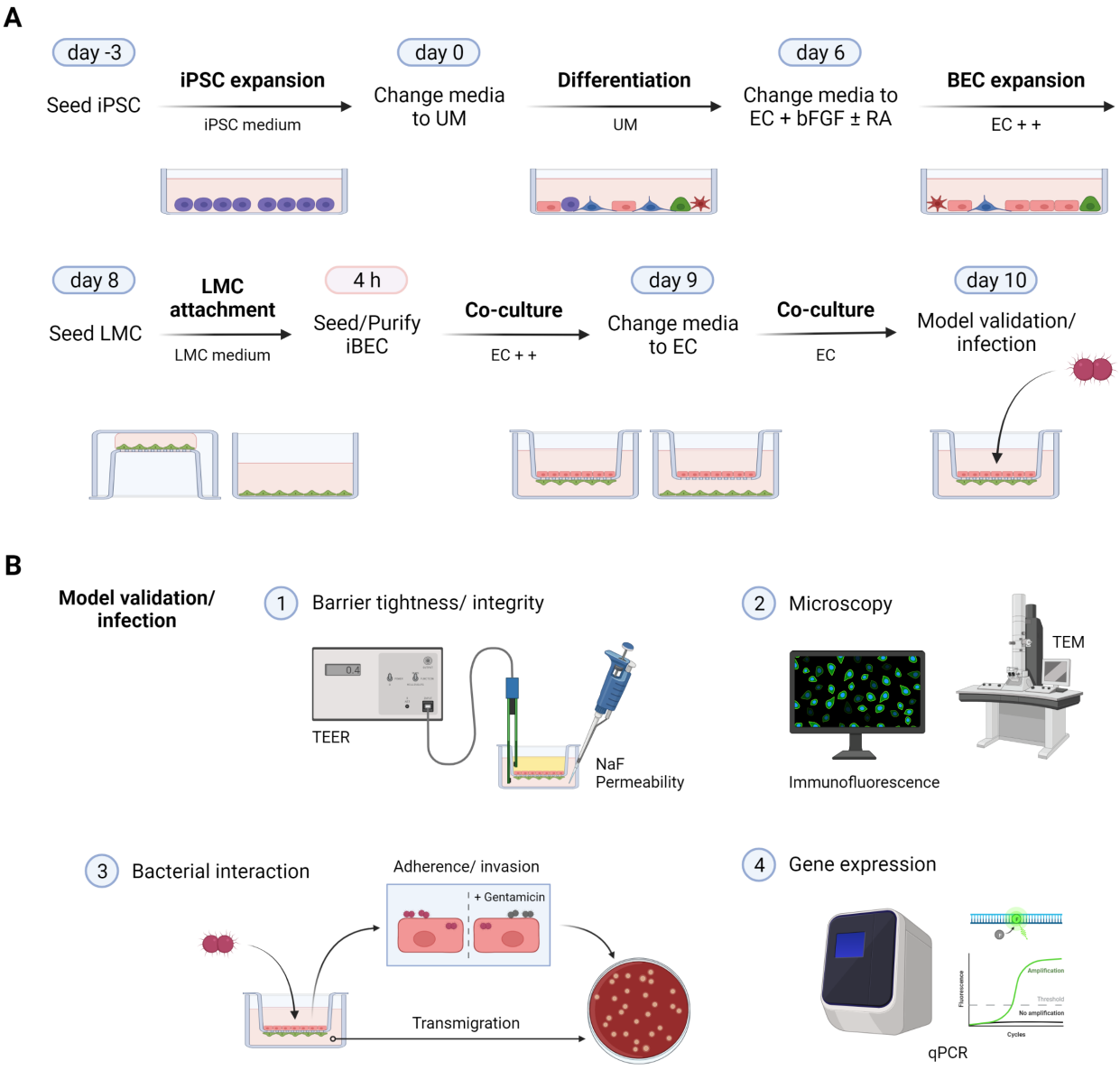
Our work highlights the usefulness of advanced in vitro models of the human mBCSFB for infection studies that more closely mimic the meningeal microenvironment. Further advancement of the model can be achieved by adding other relevant cell types such as immune cells or introducing more physiological parameters such as shear stress.

Uploaded File(s)

Supplemental Document Upload

Schematic representation of iPSC-derived brain endothelial-like cell (iBEC) differentiation and co-culture with leptomeningeal cells (LMCs).

Figure 1


A
day -3
Seed iPSC
iPSC expansion
iPSC medium
day 0
Change media to UM
Differentiation
UM
day 6
Change media to EC + bFGF ± RA
BEC expansion
EC + +
day 8
Seed LMC
LMC attachment
LMC medium
4 h
Seed/Purify iBEC
Co-culture
EC + +
day 9
Change media to EC
Co-culture
EC
day 10
Model validation/ infection
B
Model validation/ infection
1 Barrier tightness/ integrity
TEER
NaF Permeability
2 Microscopy
Immunofluorescence
TEM
3 Bacterial interaction
Adherence/ invasion
+ Gentamicin
Transmigration
4 Gene expression
qPCR