In Vitro Models of the Blood-Brain Barrier

Prof. Pierre-Olivier Couraud
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• Introduction: phenotypic characteristics of the BBB
• Reference in vitro models of the BBB
• A new model of human BBB
• Applications of in vitro models of the BBB

The Blood-Brain Barrier

Intra-cardiac injection of fluorescently-labeled dextran (10 kDa): No diffusion of the dye into the CNS across brain capillary walls

From Furuse & Tsukita

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The Blood-Brain Barrier (BBB)

Brain endothelial cells
Pericytes
Prolonged pedicle of astrocyte
Pinocytotic vesicles
Endothelial cell
Basal membrane
Astrocytes

Pericytes control CNS capillary diameter

Peppiatt et al., Nature Oct. 2006

Brain endothelial cells present a unique phenotype, characterized by a highly restrictive permeability to solutes, due to:

1. The presence of continuous intercellular tight junctions
2. The polarized expression of specific transporters
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**In vitro Culture of Brain Microvessel Endothelial Cells**

Phenotypic characterization:
- PECAM-1 (CD31), vWF, VE-cadherin (CadhA5)
- Lectin binding sites (BSi-4, UEA-1)
- Uptake of acetylated LDL
- No staining for GFAP, CD68, sm Actin
- P-glycoprotein, transferrin receptor
- Tight junction proteins: occludin, claudins, JAM, ZO-1
- Reduced drug permeability
- Trans-endothelial electrical resistance (TEER)
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**BBB In vitro Models**

- Cultured brain microvascular endothelial cells
  - Bovine cells (Cecchelli et al., de Boer et al.)
  - Porcine cells (Galla et al.)
  - Rat cells (Deli et al., Perrière et al.)

**Co-Culture of Brain Endothelial Cells with Glial Cells**

- Glial cells (rat or porcine primary cultures; astrocytes, pericytes) are co-cultured with brain endothelial cells grown on semi-permeable filters (Transwell)

**In vitro Permeability Assay**

- Clearance(µL) = [concentration abluminal] × [volume abluminal] × [concentration luminal]⁻¹
- The average volume cleared is plotted versus time
- Permeability × surface area product value for endothelial monolayer (PSe) is: PSe endothelial⁻¹ = PSe time-plotted⁻¹ - PSe insert⁻¹
- PSe divided by the surface area is the endothelial permeability coefficient [Pe (cm/s)]

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Trans-Endothelial Electrical Resistance (TEER)

- Chopstick electrodes with the Millicell ERS® voltmeter: easy to use, but hardly reproducible
- Endohm system: large areas to cover the whole filter, more reproducible
- Impedance spectroscopy: resistance and capacitance measurements (H.U. Galla’s group, Germany) cells are grown either on insert filters or directly on gold micro-electrodes
  - Coculture with astrocytes: Enhanced TEER from 50-100 to 500-1000 Ω.cm²

Correlation Between In vitro and In vivo Drug Permeability

Comparison of various In vitro models
In vitro models for the blood brain barrier

Correlation between distribution to brain tissue in vivo and transport across BBEC in vitro

Correlation between distribution to brain tissue in vivo and transport across Caco-2 cells in vitro

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Immortalization of Rat Brain Endothelial Cells

- RBE4 (E1A) - Roux et al. (1994)
- GP8 (T-SV40) - Greenwood et al. (1996)
- GPNT (T-SV40) - Regina et al. (1996)
- TR-BBBS (T-SV40) - Terasaki et al. (2001)
- rBCEC4 (Polyoma T) - Blasig et al. (2001)

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- rBCEC4 (Polyoma T)

BBB In vitro Models:
Primary BMECs vs. immortalized cell lines

Primary BMECs
- Bovine cells, Porcine cells
  - Very good differentiation
  - Limited supply
  - Limited life-span

Rat immortalized cell lines
- Good differentiation
- Unlimited supply
- Unlimited life-span
- Susceptible to genetic engineering

Towards an in vitro model of human BBB?

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Immortalization of Human Brain Endothelial Cells
- Infection of primary cultures by lentiviral vectors encoding hTERT and T-SV40
- Selection of fully differentiated cellular clones

hCMEC/D3: A Non-Transformed Phenotype (1)
- Contact-inhibited, substrate- and growth factor-dependent proliferation
- Normal diploid phenotype
- Absence of proliferation in soft agar

hCMEC/D3: A Non-Transformed Phenotype (2)
Formation of capillary-like structures in 3D gels
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hCMEC/D3: Expression of Junction Proteins (1)

hCMEC/D3: Expression of Junction Proteins (2)

Correlation Between In vivo and In vitro Permeability
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**Efflux Pumps: ABC Transporters**

- Multidrug resistance protein: MDR-1 / ABCB1
- Multidrug resistance-related proteins: MRP-1 / MRP-7 / ABC17
- Breast cancer resistance protein: BCRP / ABCG2

**hCMEC/D3: Functionality of ABC Transporters**

- Drug uptake (% control)
  - Calcein-AM
  - Rhodamine 123
  - [3H] Daunorubicin

**The hCMEC/D3 Cell Line**

- Stably maintains a non-transformed endothelial phenotype, with growth factor-dependent proliferation, contact-inhibition.
- Expresses key tight junction-associated proteins known to be expressed at the BBB.
- Expresses the expected adhesion molecules (ICAM-1, VCAM-1, etc.) and chemokine receptors involved in leukocyte infiltration across the BBB.
- Displays a restricted permeability to small hydrophilic molecules.
- Expresses functional ABC transporters: P-gp (MDR-1), MRP, BCRP.

We believe that the hCMEC/D3 cell line may constitute a unique in vitro model of human BBB.
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The hCMEC/D3 Model Under Flow: Further Differentiation
hCMEC/D3 in a DIV-BBB device
(Collaboration with Dr. D Janigro, Cleveland, USA)

hCMEC/D3 in a DIV-BBB Device
hCMEC/D3 cells present high TEER, even without astrocytes

Hyperosmolar Opening of the BBB in DIV Models
Transient TEER decrease induced by intraluminal perfusion (30 sec) with hyperosmolar mannitol (1.6M)
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[14C]-Sucrose Permeability in DIV Models

hCMEC/D3 cells display a very low permeability to sucrose:

2.11 x 10^{-7} \pm 0.53 \text{ cm/sec}

i.e., 1.3 x 10^{-5} \text{ cm/min} vs. 1.6 x 10^{-3} \text{ cm/min (Transwell)}

hCMEC/D3 in a DIV-BBB Device

Conclusions

hCMEC/D3 cells have the intrinsic capacity to display a BBB phenotype, when grown in appropriate conditions

“Imortalized human brain endothelial cells and flow-based vascular modeling: a marriage of convenience for rational neurovascular studies”

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**BBB Physiological and Pathophysiological Roles**

- What is the cellular and molecular basis of the BBB?
- CNS inflammation: what is the functional role of brain endothelium in leucocyte, virus or bacteria infiltration?
- How is brain endothelium involved in CNS tumor progression?
- Brain vasculature dysfunctions in stroke?
  - How can we deliver therapeutic drugs to the CNS through the BBB?
  - How can we design and assay new drug candidates?

**In vitro BBB Models and Research Axes**

- Mechanisms of brain endothelial response to cell adhesion and transmigration: endothelial cell signaling, regulation of BBB permeability
- Structural and functional analysis of tight junctions, receptors, transporters: drug targeting to the CNS
- High throughput screening of drug candidates: drug discovery

**Brain Endothelial Response to Cell Adhesion and Transmigration**

Leukocyte infiltration across the BBB

- **Rolling**
  - Selectin ligands, α4-integrins

- **Firm adhesion**
  - β2-integrins, α4-integrins

- **Infiltration**
  - ICAM-1, -2, VCAM-1

- **Tissue retention**
  - β1-integrins

- E- and P-selectins, VCAM-1

- ECM: ICAM-1, PECAM-1, CD99, JAM-1, CD40, HA
Brain Endothelial Response to Cell Adhesion and Transmigration

- Leucocyte migratory cup or docking structure for leukocyte firm adhesion and trans-endothelial migration

Brain Endothelial Response to Cell Adhesion and Transmigration

- ICAM-1 and PECAM-1 signalings control trans-endothelial migration of leukocytes

Tight Junctions, Receptors and Transporters

- Differential global proteomic analysis by 2DE - DIGE / MS-MS (Coll. R. Cecchelli & C. Fihaut, LENS, France)
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Tight junctions, receptors and transporters
Analysis of TJ Proteomes in hCMEC/D3 Cells

A key role for Claudin-5 at the blood-brain barrier

Impairment of the blood-brain barrier in Claudin-5-deficient mice

Proteomics: Absolute Quantification of Membrane Proteins by Multiple Reaction Monitoring (MRM)

Select the best peptide candidate of protein of interest by in silico calculation

Optimize LC-MS/MS separation protocol for best quantitation

Add known quantity of synthetic peptide (internal standard) to sample

Trypsin digest

Reproduce LC-MS/MS separation protocol to perform absolute quantitation (integrate areas on mass spectrum)

Proteomic Analysis of BBB Transporters and Receptors by Quantitative Mass Spectrometry

In plasma membrane fraction of hCMEC/D3 cell (fmol/mg protein)

Mouse brain capillary fraction (fmol/mg protein)

Glut1

Na+/K-ATPase

Mdr1a / MDR1

Bcrp / BCRP

Mrp4 / MRP4

Mrp1 / MRP1

Mct1 / MCT1

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Tight junctions, receptors and transporters
Optimizing Drug Delivery to the Brain

- Can we modulate ABC-transporters activity?
- Can we take advantage of the internalization of receptors/transporters on brain endothelium? i.e., TransR, p97, HB-EGF
- Can we identify new markers of brain endothelium?
- Can we design new vectors targeted to brain endothelium?

1. Tight junction structural and functional organization
2. Cellular/molecular analysis of the «neurovascular unit» identification of BBB-inducing factors and regulators
3. Understanding BBB dysfunction in CNS pathologies: stroke, multiple sclerosis, Alzheimer's disease
4. Optimizing drug delivery to the brain

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