Role of pericytes in the induction of BBB properties

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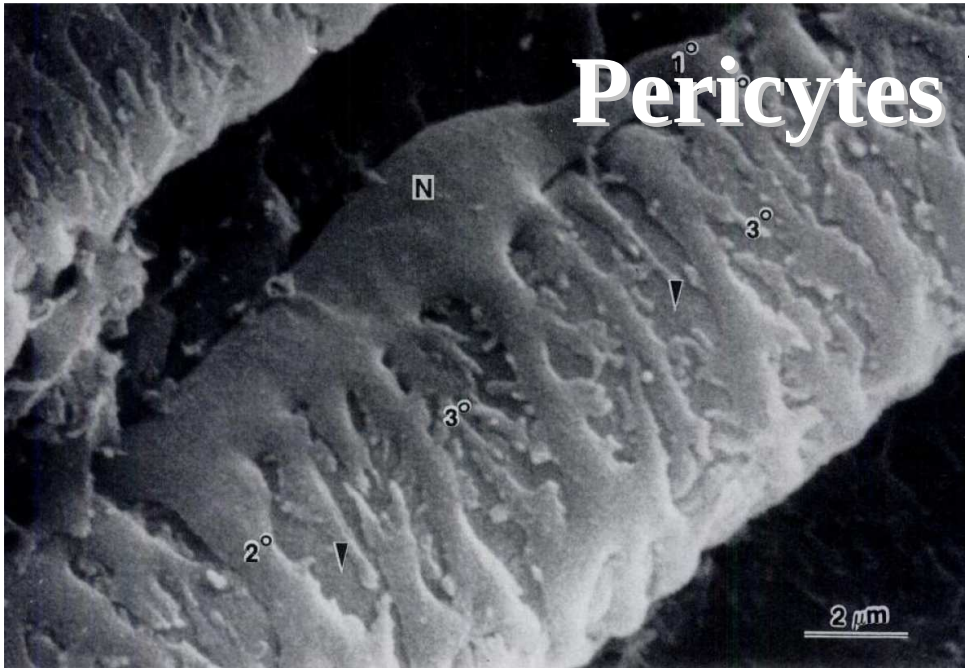
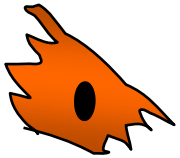
Pericytes background



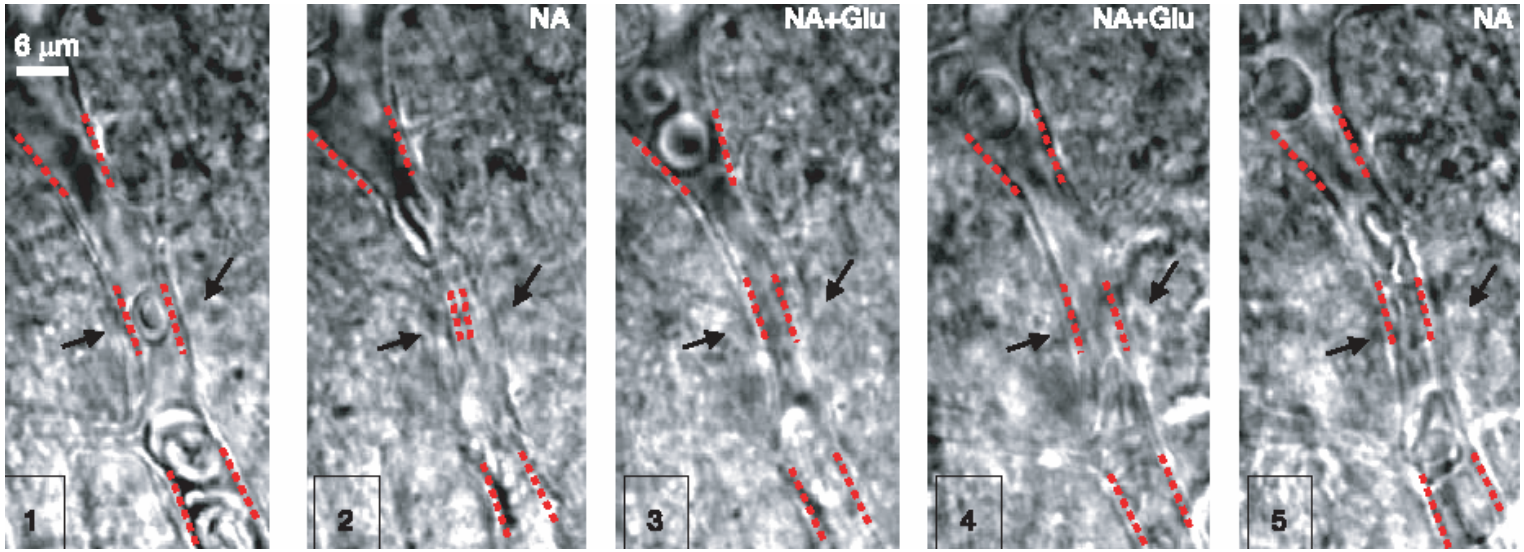
SEM: Shepro and Morel, FasebJ, 1993

- Cells of mesodermal origin that envelop microvessels
- Morphological, biochemical and physiological heterogenity
- In situ ratio of pericytes to endothelial cells
  - ***cell:cell ratio***
    highest in retina (1:1-1:3) and brain (1:5), lowest in muscle (1:100) and glands
  - ***pericyte:endothelial cell area ratio***
    highest in retina (0.9-1.1) and brain (0.6-0.8)
  - related to
    the degree of tightness of the interendothelial junctions
    the level of microvascular blood pressure

Pericytes: functions



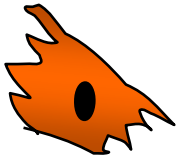
- Regulate endothelial proliferation and differentiation
- Contractile cells; regulate blood flow & vessel permeability



Peppiatt et al., Nature, 2006

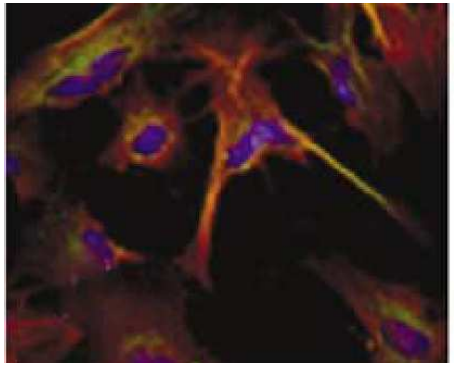
- Pericytes function as progenitor cells
- Synthesize and secrete
  - vasoactive mediators and regulators
  - basement membrane and extracellular matrix components
- Involved in microvascular diseases

Brain pericytes: characteristics

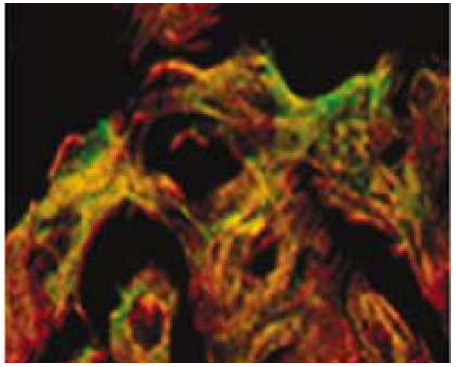


- Markers
  - α-SM actin, nestin, NG2
  - CD11b, Thy1.1
  - vimentin, aminopeptidase-N
  - endosialin

α-SMA/nestin



α-SMA/NG2

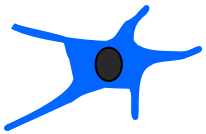


Dore-Duffy et al., JCBFM, 2006

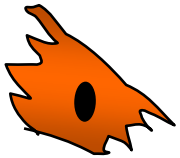
- Express
  - Receptors: PDGF-Rβ, TGF1β, VEGF-R (Flt1), Tie-2, AT1A
    mGlu-R1α, β2AR
  - Transporters: MRP1, MRP5 (but not P-glycoprotein)
- Constitutively synthesize angiopoietin 1
- Adult CNS microvascular pericytes exhibit neural stem cell activity (pericytes, neurons, and glial cells)
- Regulate brain angiodynamics (Dore-Duffy and La Manna, 2007)

In vitro models: induction of BBB properties

- in vivo BBB characteristics are induced by organ-specific signals
- BBB phenotype is lost in cultures without induction
- **Astroglia** cells increase BBB properties:
  - strengthen the barrier
  - increase the expression and activity of enzymes and transporters
  - used in great majority of co-culture BBB models

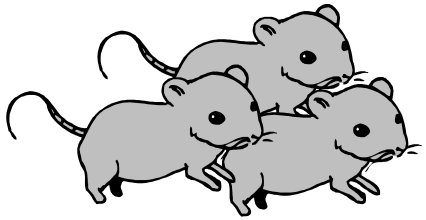


- **Neurons** increase BBB properties; few data
- **Pericytes**



  - pericytoma: no change in TEER
  - bovine retina pericyte-CM: decrease in TEER (Raub et al., 1992)
  - primary rat pericytes
    - increased TEER (Hayashi et al., 2004)
    - decreased permeability in MBEC4 cells (Dohgu et al., 2005)
    - up-regulated endothelial MRP6 (Berezowski et al., 2004)
  - soluble factors: TGF-β (Dohgu et al., 2005)
    - angiopoietin-1 (Hori et al., 2004)

Preparation of primary cultures



Rat brain

Rat brain

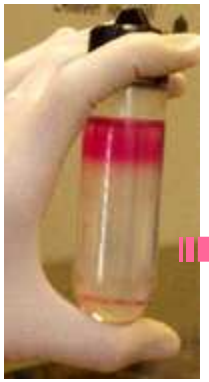
isolation of microvessel fragments

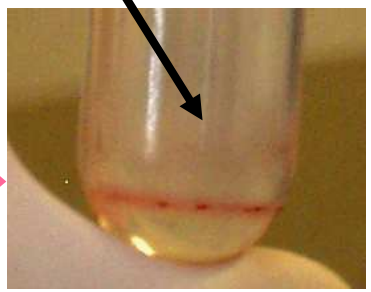
“Shaking” method

astrocytes

pericytes

microvascular endothelial cells

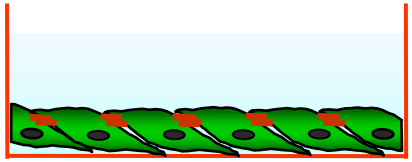


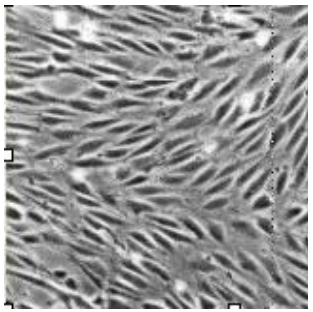


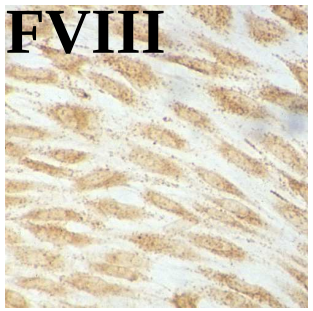
layer of MV fragments

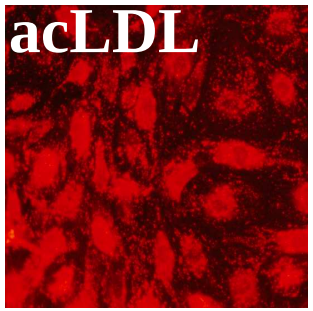
Percoll gradient separation

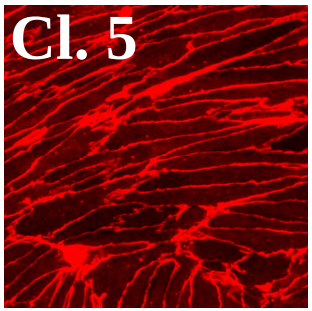
Primary cell cultures used for the BBB models

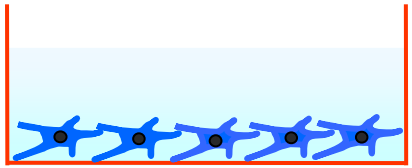


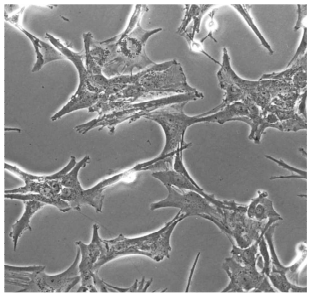


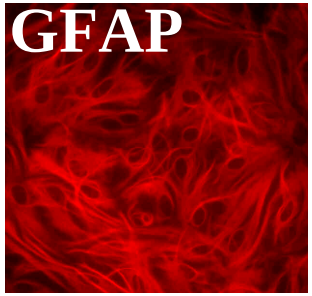


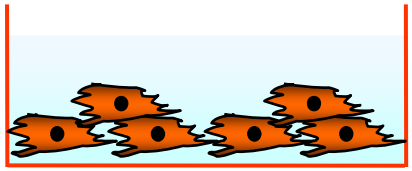


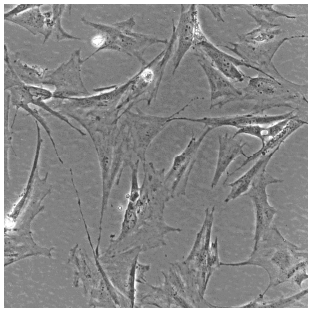


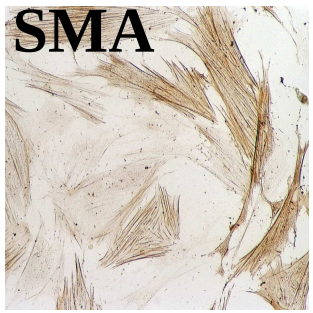


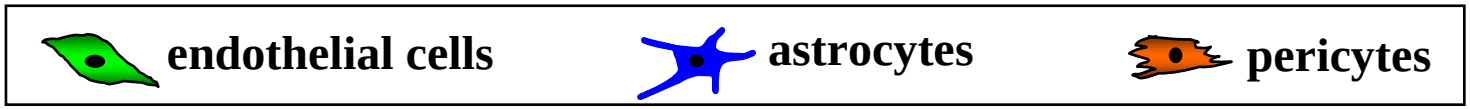




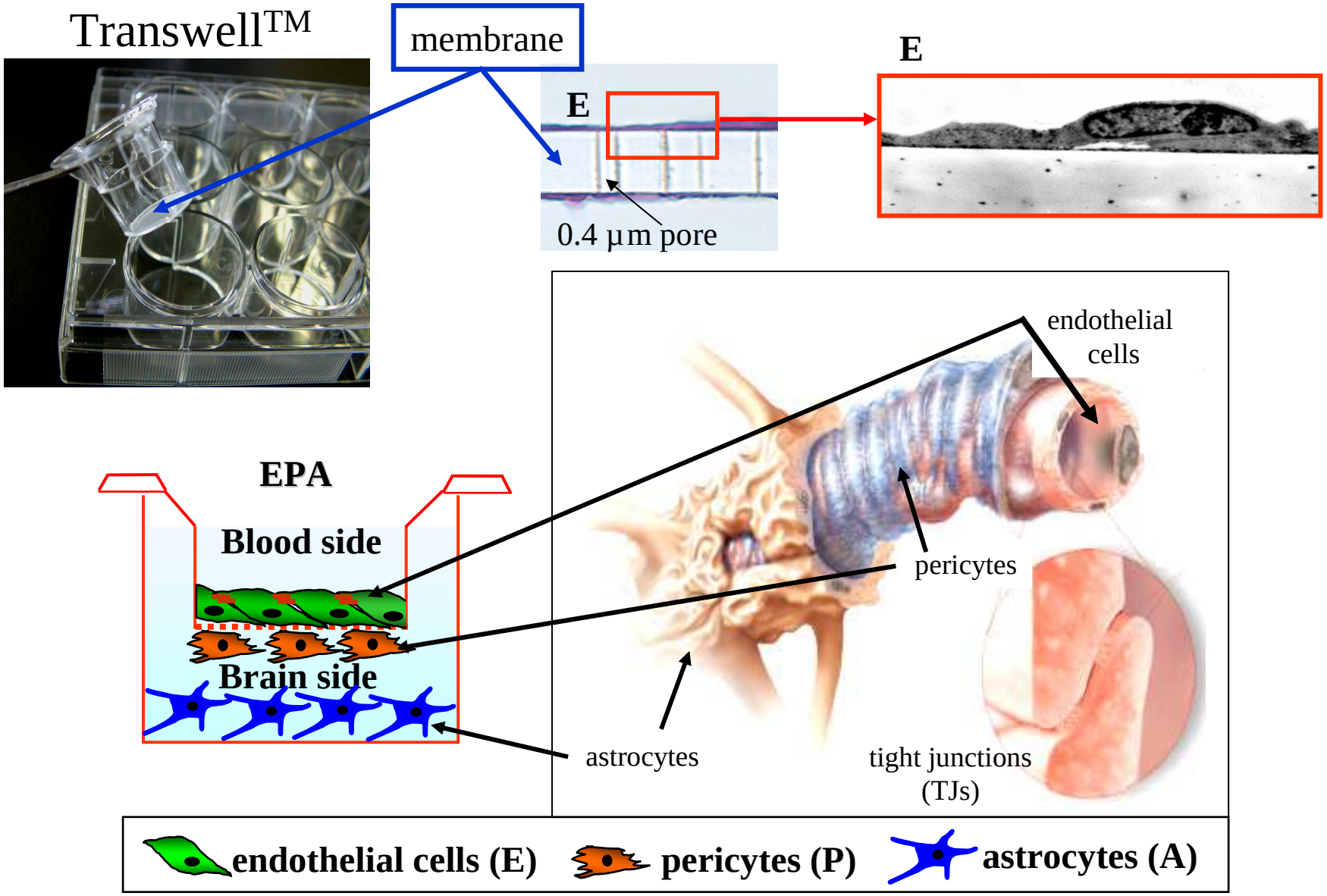






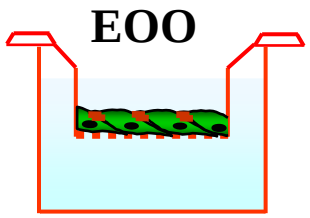


Co-culture of three cell types

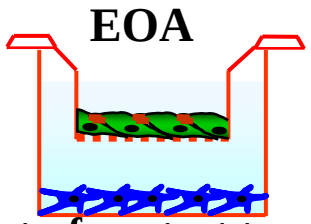


Scheme of the BBB models

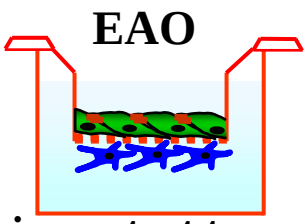
Mono-culture
Endothelial cells (E)



Double co-cultures
with astrocytes (A)

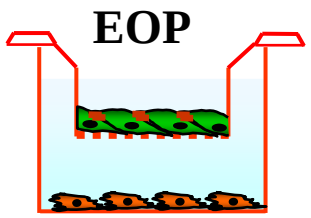


out-of-contact type

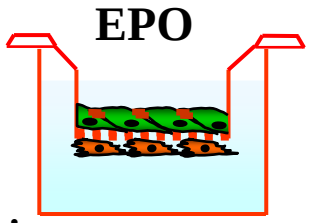


in-contact type

with pericytes (P)

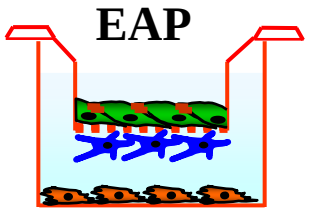


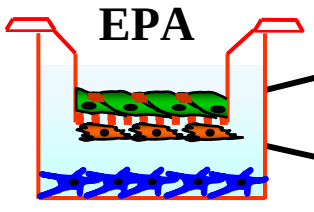
out-of-contact type

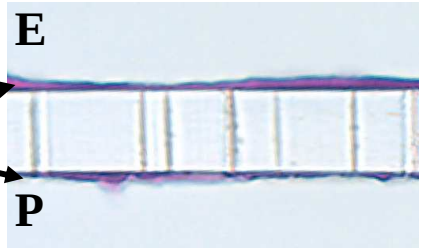


in-contact type

Triple co-cultures
with pericytes and astrocytes

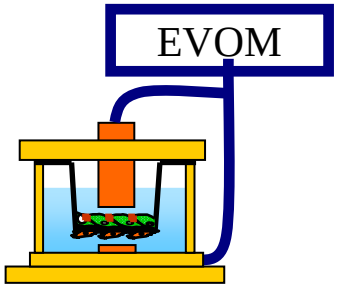


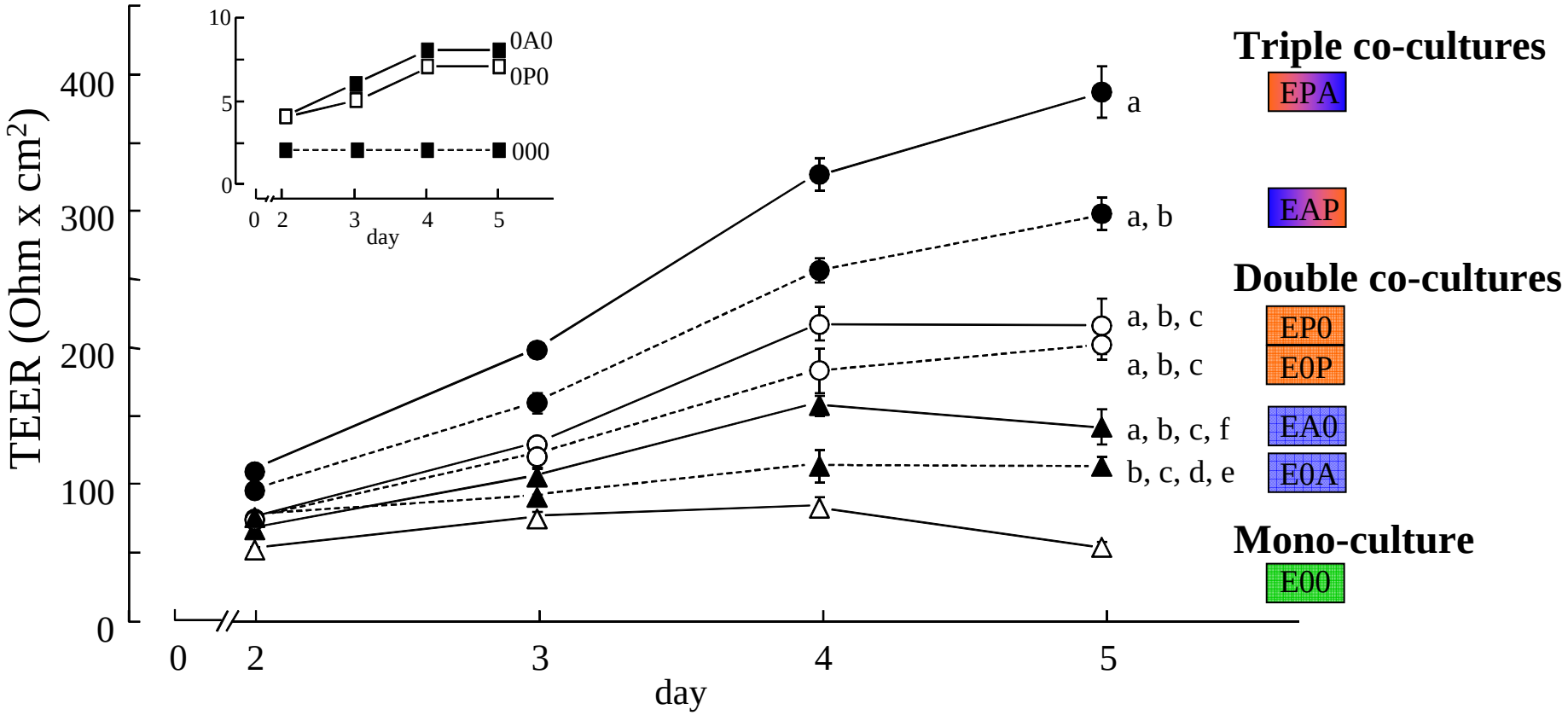




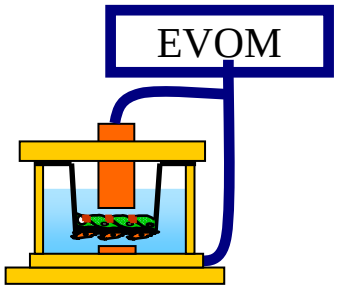
endothelial cells (E) **pericytes (P)** **astrocytes (A)** **no cell (O)**

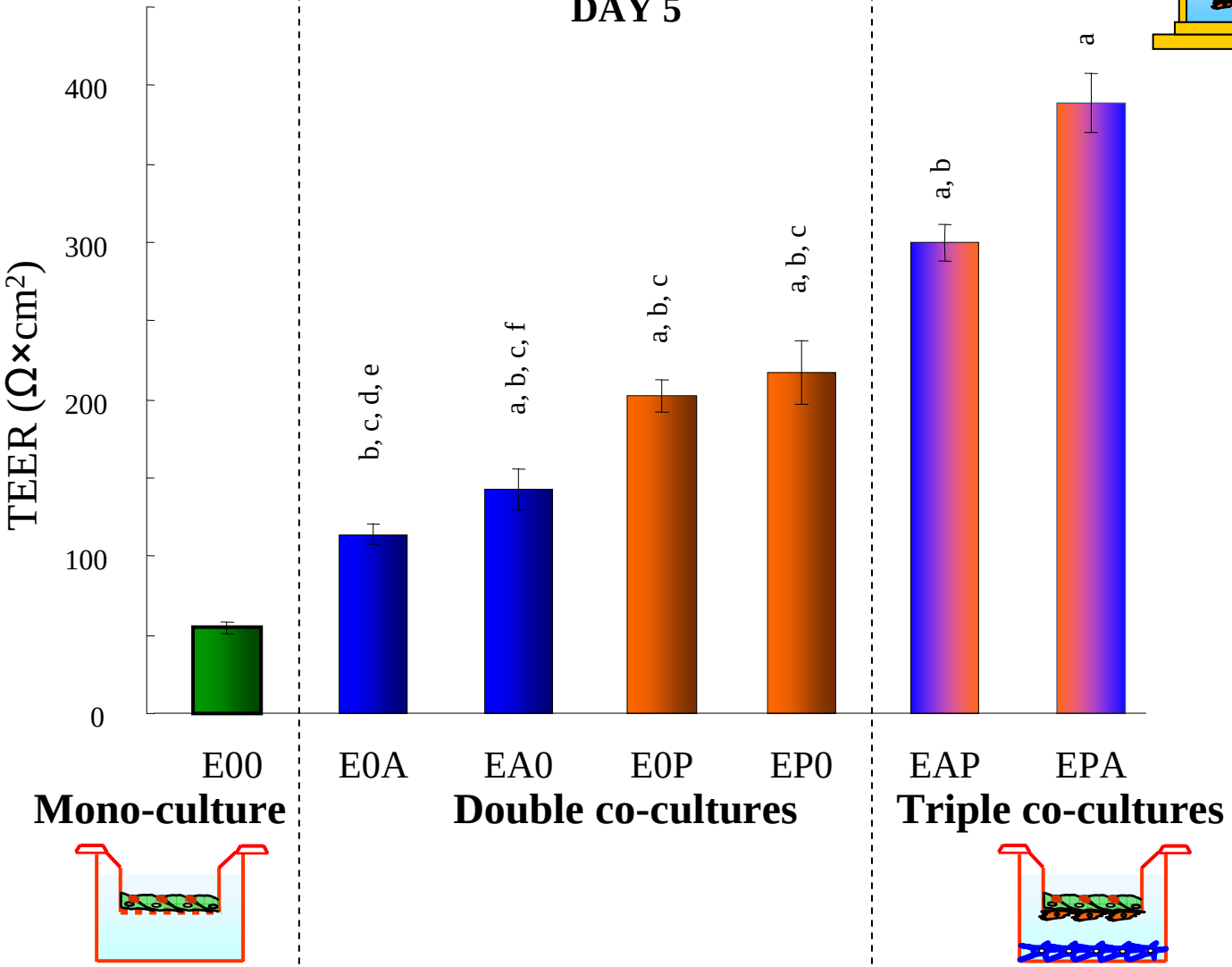
Effect of co-culture on TEER





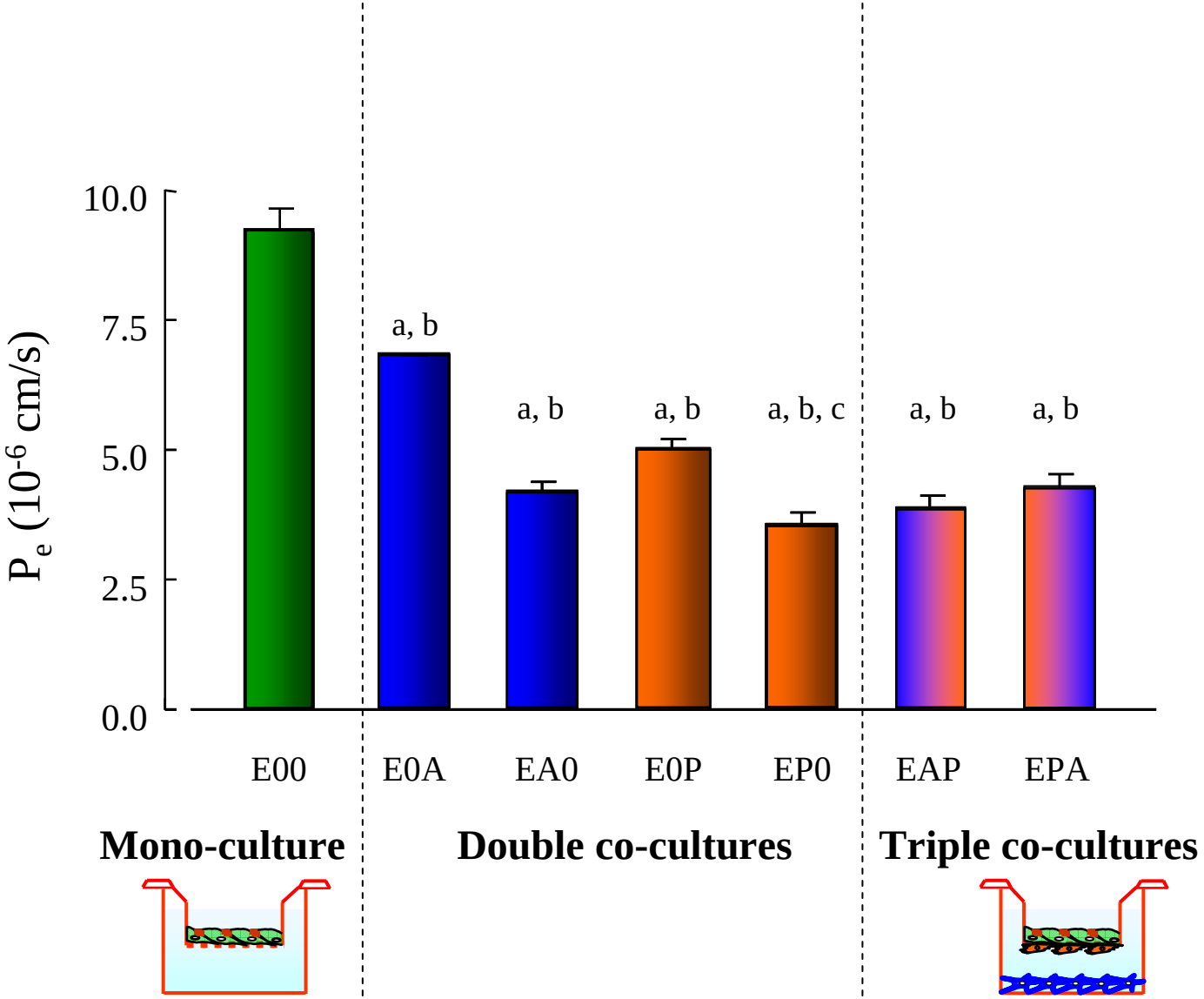
Effect of co-culture on TEER





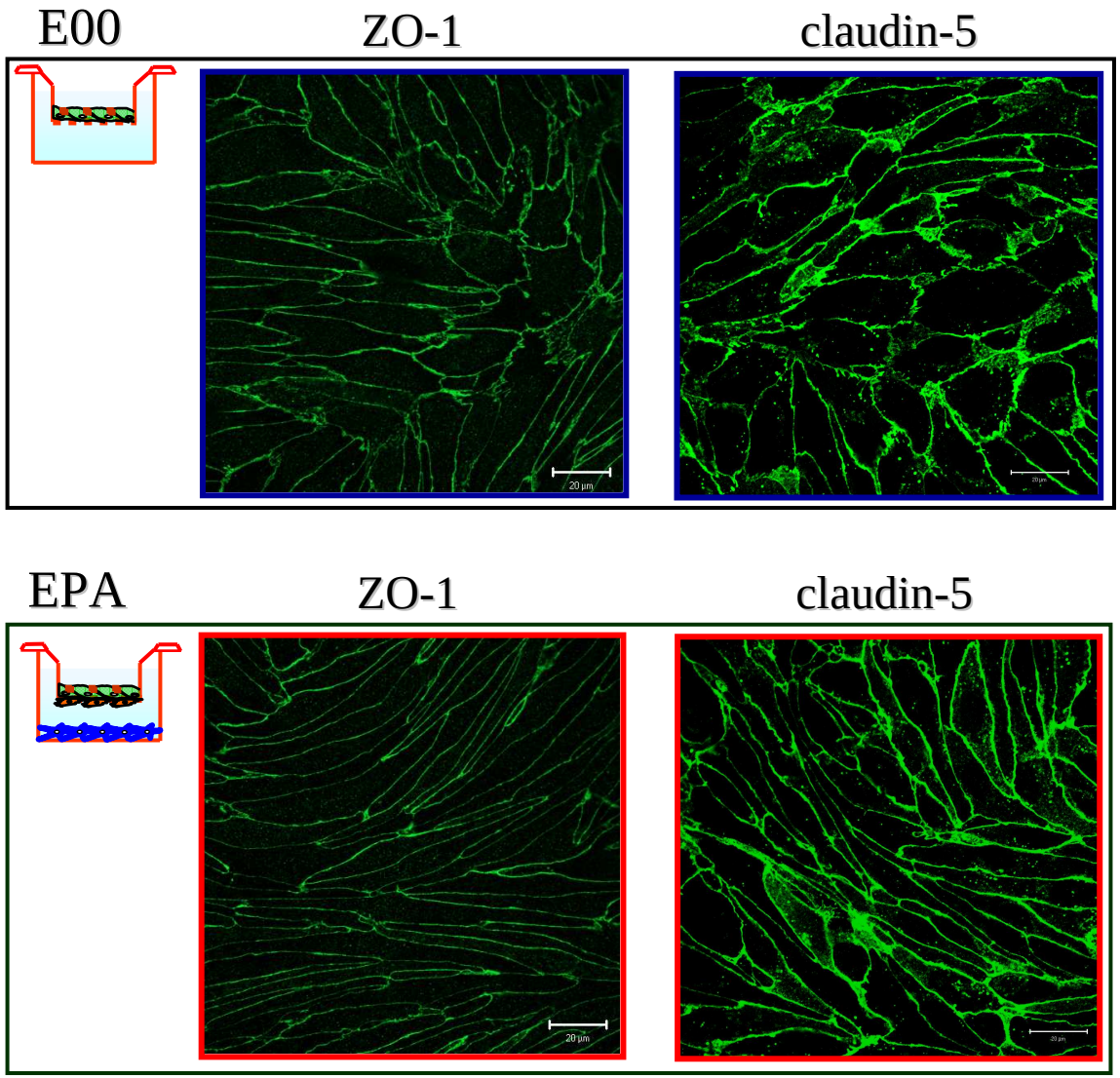
[a]$p < 0.01$ vs. E00 [b]$p < 0.01$ vs. EPA [c]$p < 0.01$ vs. EAP [d]$p < 0.01$ vs. EP0 [e]$p < 0.01$ vs. E0P [f]$p < 0.05$ vs. EP0

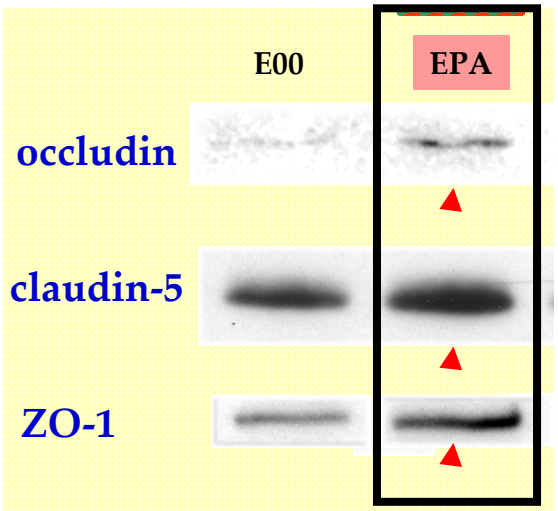
Effect of co-culture on fluorescein permeability



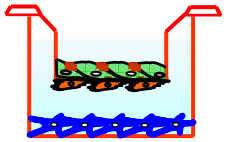
[a]$p < 0.01$ vs. E00 [b]$p < 0.01$ vs. EOA [c]$p < 0.01$ vs. EOP

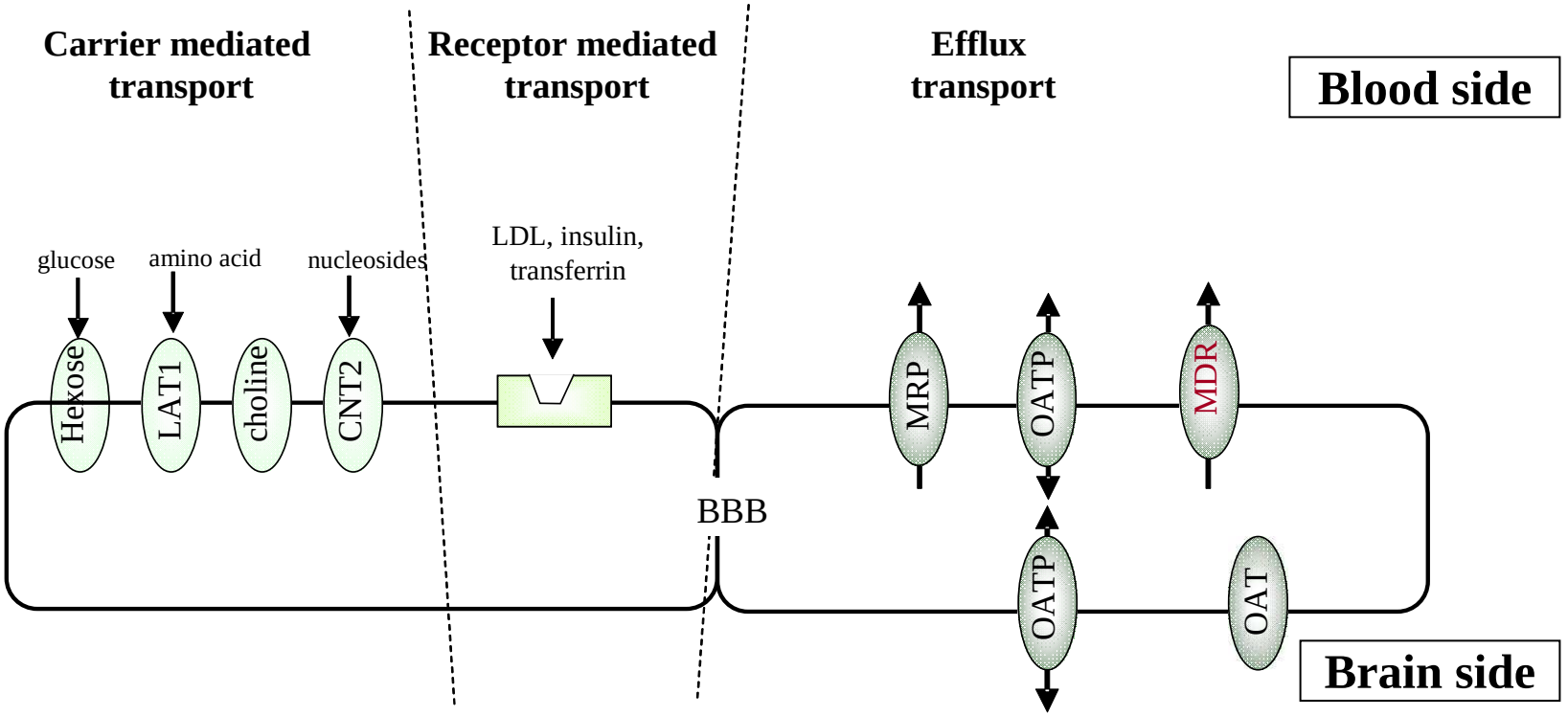
Triple co-culture and tight junction proteins



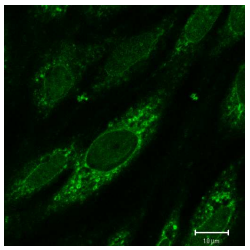


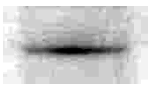
Triple co-culture and transporters



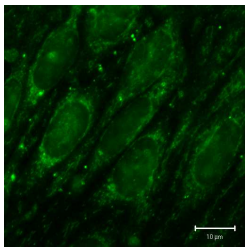


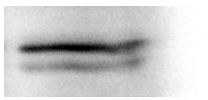
GLUT1



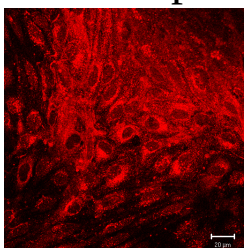


RAGE

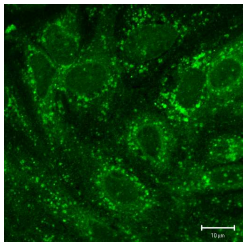




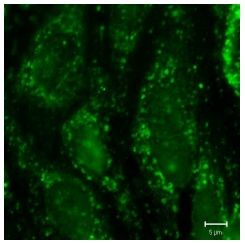
LDL receptor

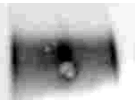


MRP1



MDR

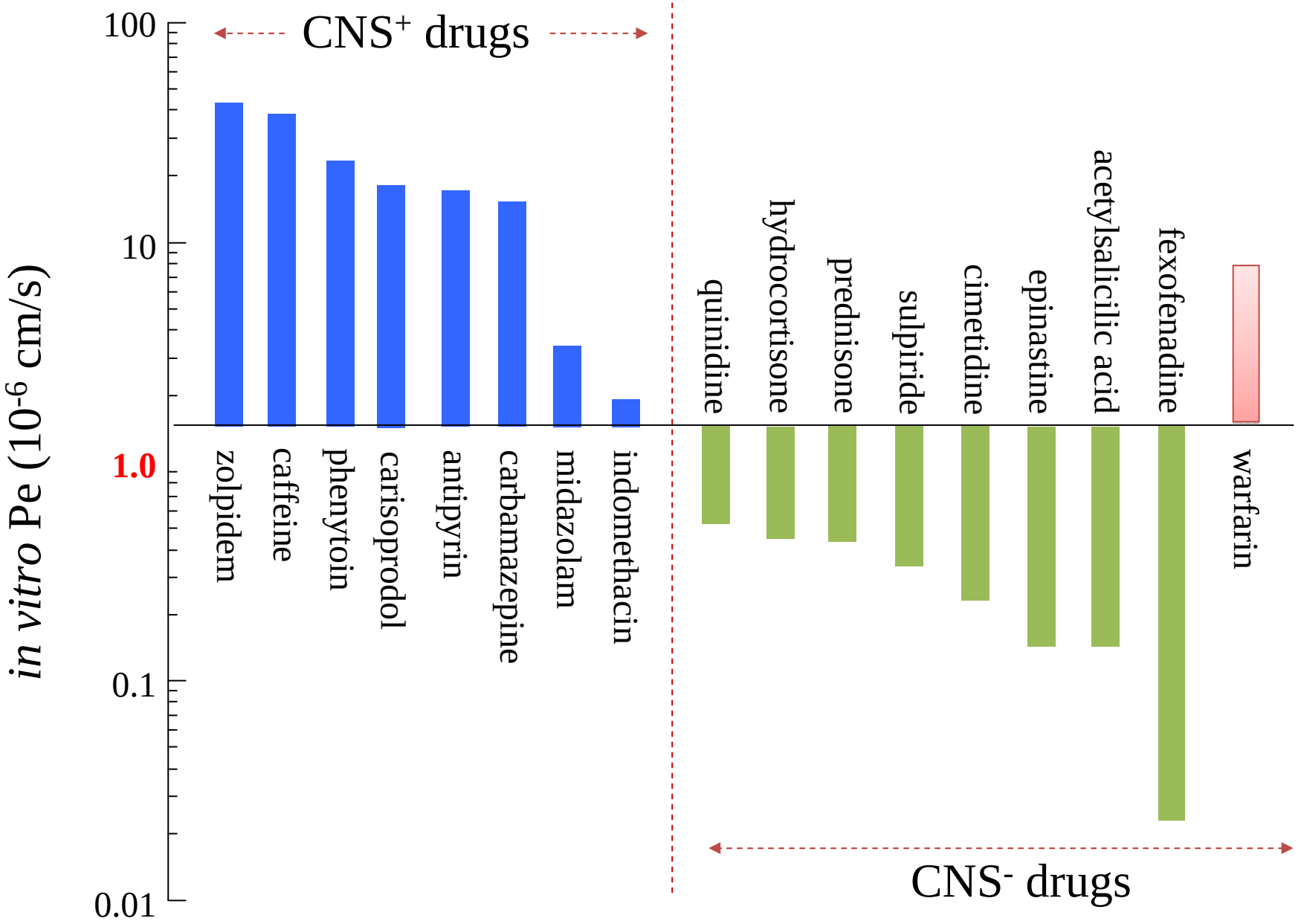




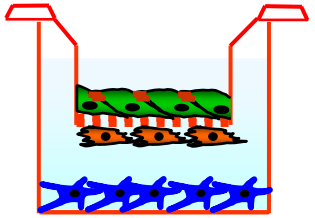
Drugs selected for permeability studies

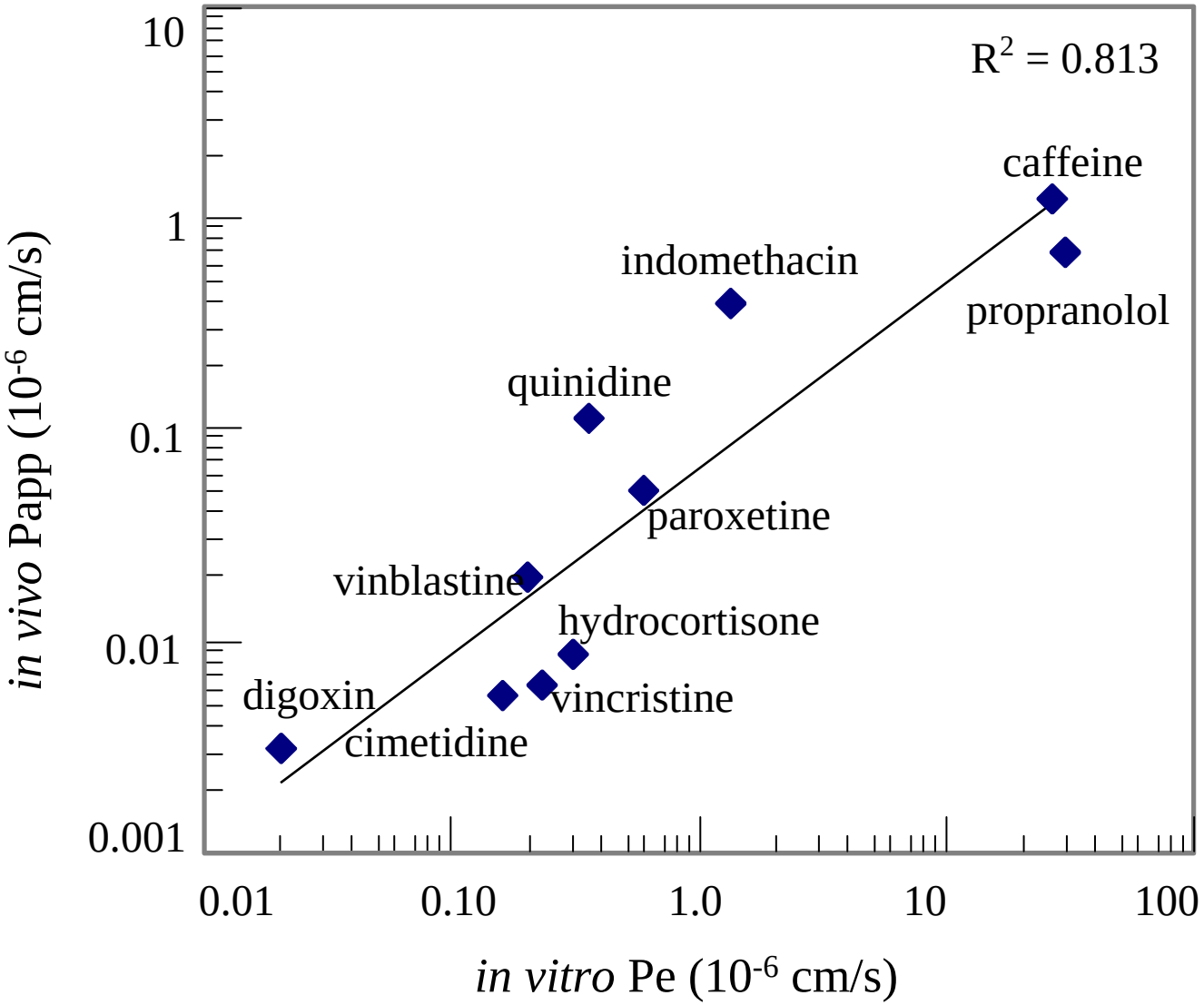
name	MW	CNS	transport	Recovery rate (%)
zolpidem	382	+	passive lipophilic	71.7
caffeine	212	+	passive lipophilic	87.5
phenytoin	252	+	lipophil and high protein binding	99.6
carisoprodol	260	+	passive lipophilic	89.6
antipyrin	188	+	passive lipophilic	90
carbamazepine	236	+	passive lipophilic	85.1
midazolam	326	+	passive lipophilic, highly permeable, Pgp substr.	84.2
indomethacin	358	+	passive hydrophilic	87.6
propranolol	296	+	passive lipophilic	81.9
quinidine	783	-	efflux	72.8
hydrocortisone	362	-	efflux (Pgp)	88.2
prednisone	358	-	efflux (Pgp)	93.2
sulpiride	341	±	efflux: Pgp, influx: OCTN1, OCTN2, PEPT1	91.7
cimetidine	252	-	efflux	95.4
epinastine	286	-	efflux	94
acetylsalicilic acid	180	-	organic anion, efflux (Oat1, Oat2)	94.2
fexofenadine	538	-	efflux: Pgp, OATP1A2	93.1
warfarin	346	-	lipophil and high protein binding	79.8

Drug permeability of the triple co-culture BBB model

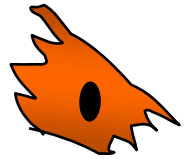


Comparison of drug permeability BBB model vs. *in vivo* data

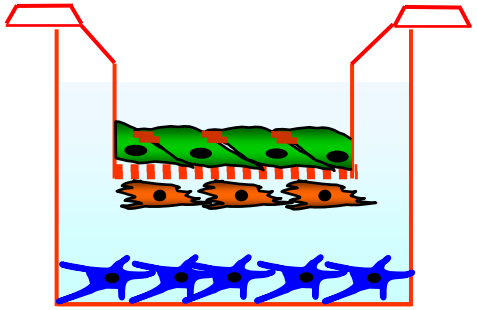




Summary



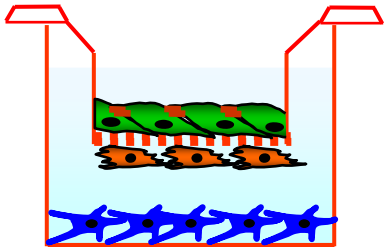
- Brain microvessel pericytes were as effective as astroglia to induce BBB properties in primary rat brain endothelial cells
- Pericytes could further enhance the inductive effect of astroglia
- The in-contact models were tighter than the out-of-contact types
- Triple models showed better barrier properties than double co-cultures



- The first syngeneic primary culture-based rat BBB model using pericytes mimicking in vivo anatomical situation

Nakagawa et al., Cellular and Molecular Neurobiology, 2007, epub

Acknowledgement



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