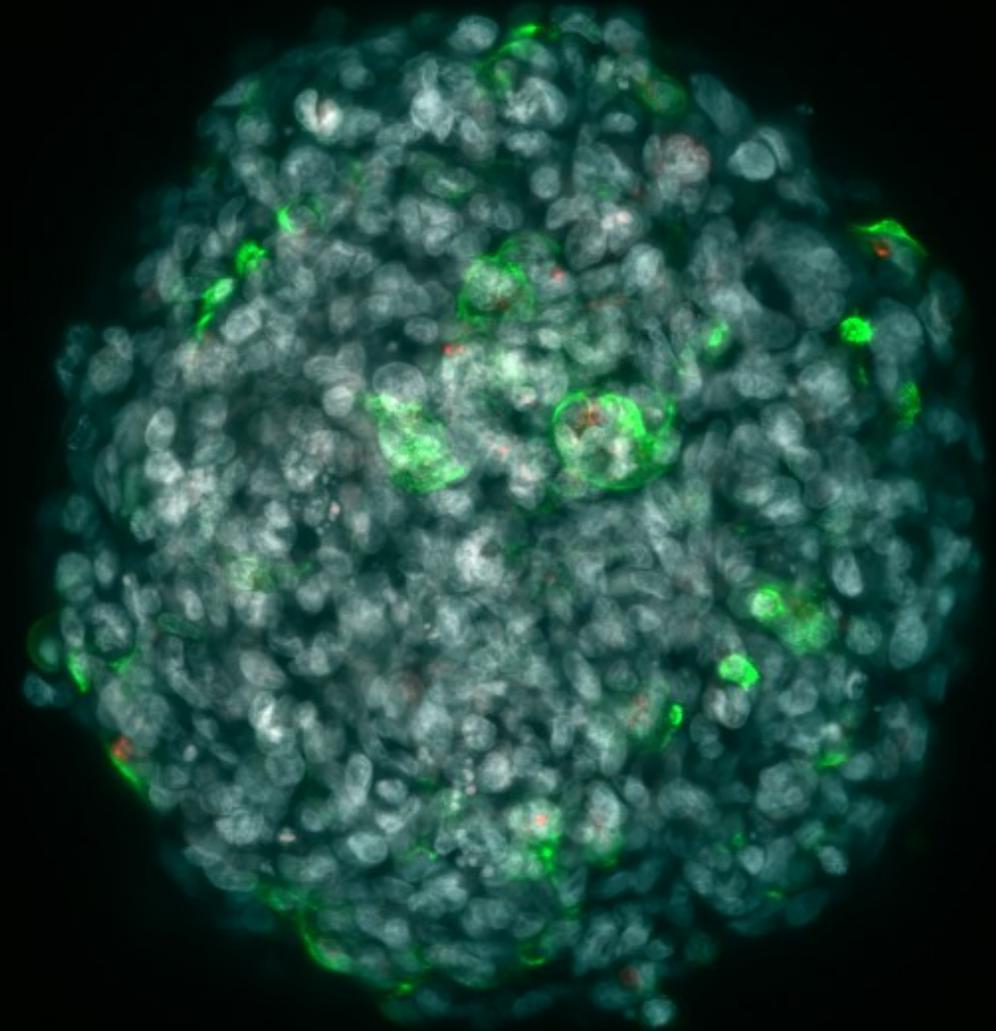


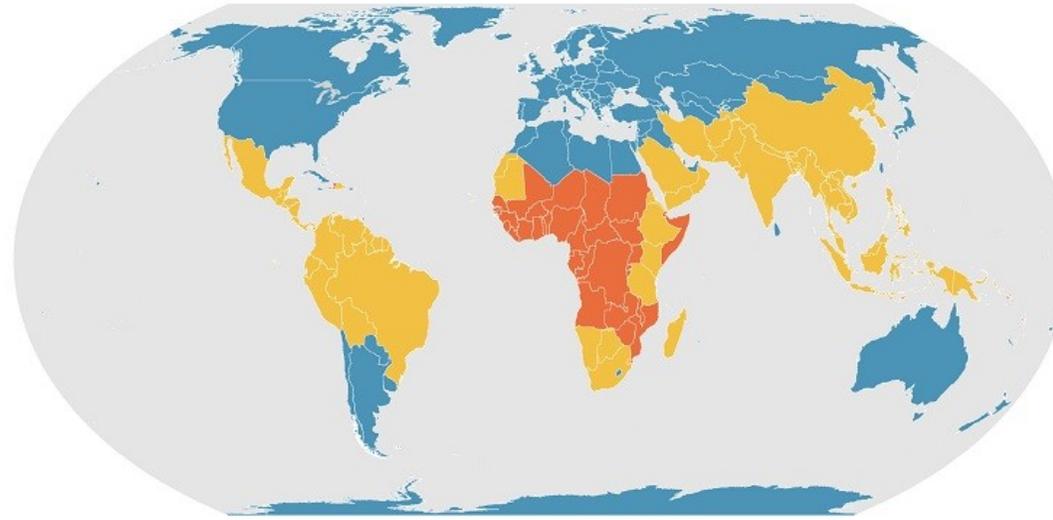
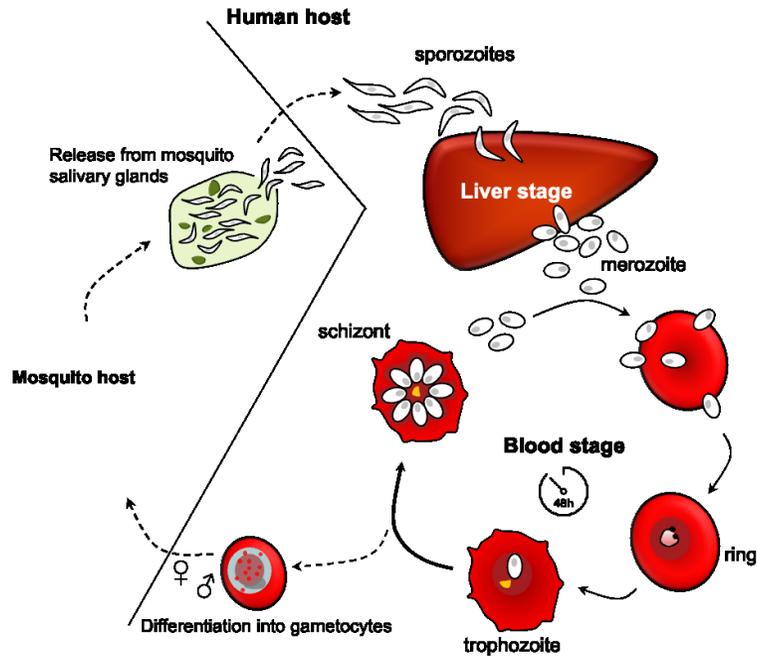
Human derived blood-brain barrier organoids to study brain infections

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UNIVERSITY OF COPENHAGEN



Malaria



■ Malaria transmission is not known to occur
■ Malaria transmission occurs in some places
■ Malaria transmission occurs throughout

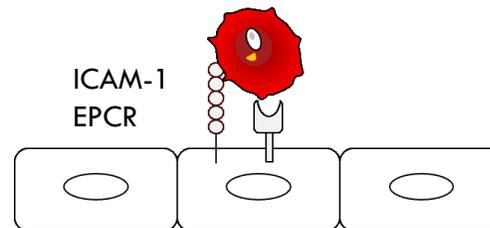
Most at Risk



Multiple Organs Affected

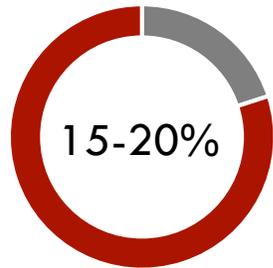


Cerebral Malaria



Neurological Complications

- loss of motor function
- learning impairment
- increased risk of epilepsy



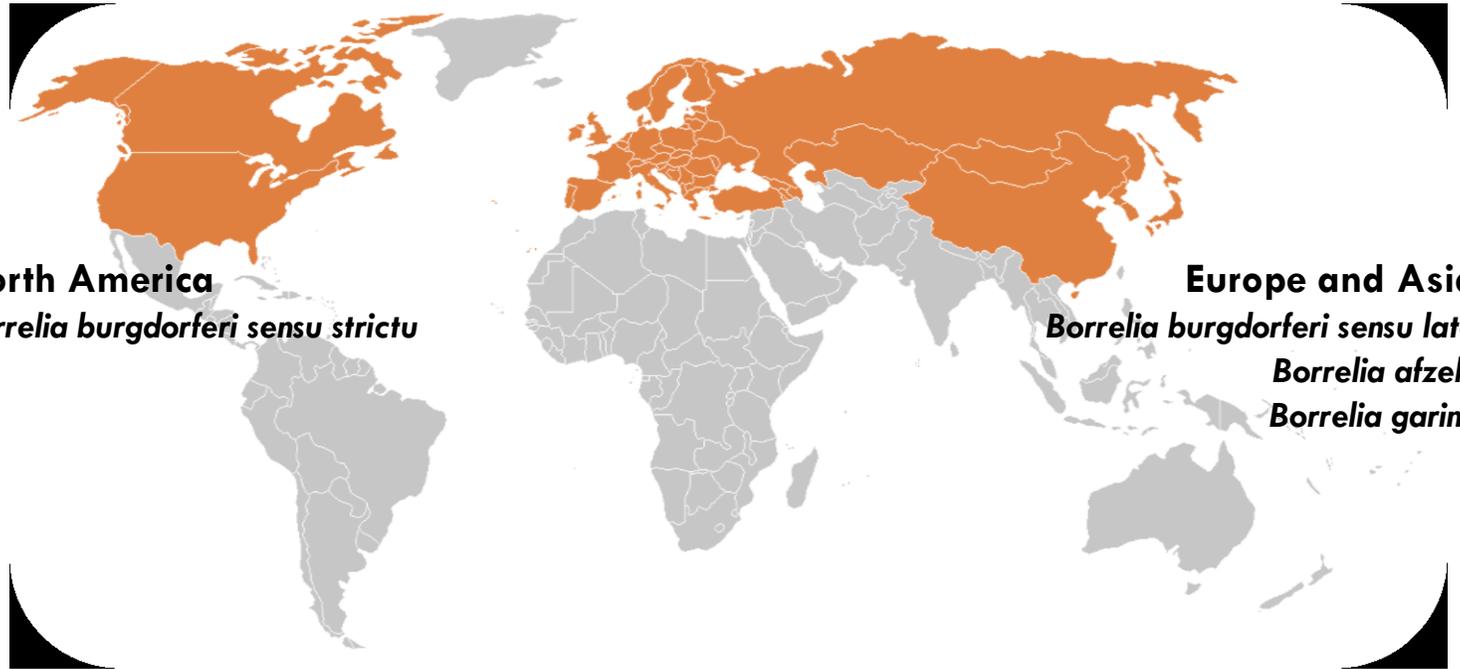
Mortality Rate

Neuroborreliosis

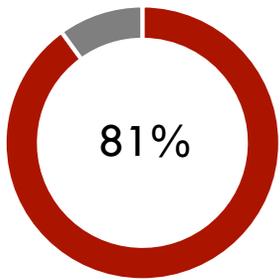


North America
Borrelia burgdorferi sensu strictu

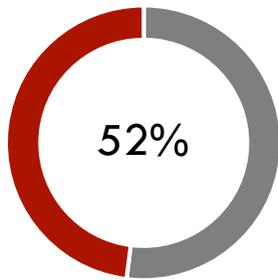
Europe and Asia
Borrelia burgdorferi sensu lato
Borrelia afzelii
Borrelia garinii



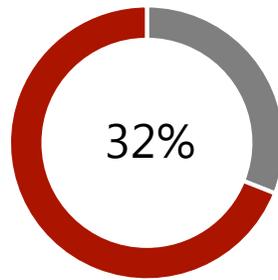
Symptoms



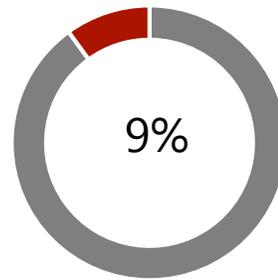
Radiculoneuritis



Facial Palsy



Headache



Double Vision

Treatment

oral or IV antibiotics

Doxycycline
Amoxycillin
Azithromycin
Ceftriaxone

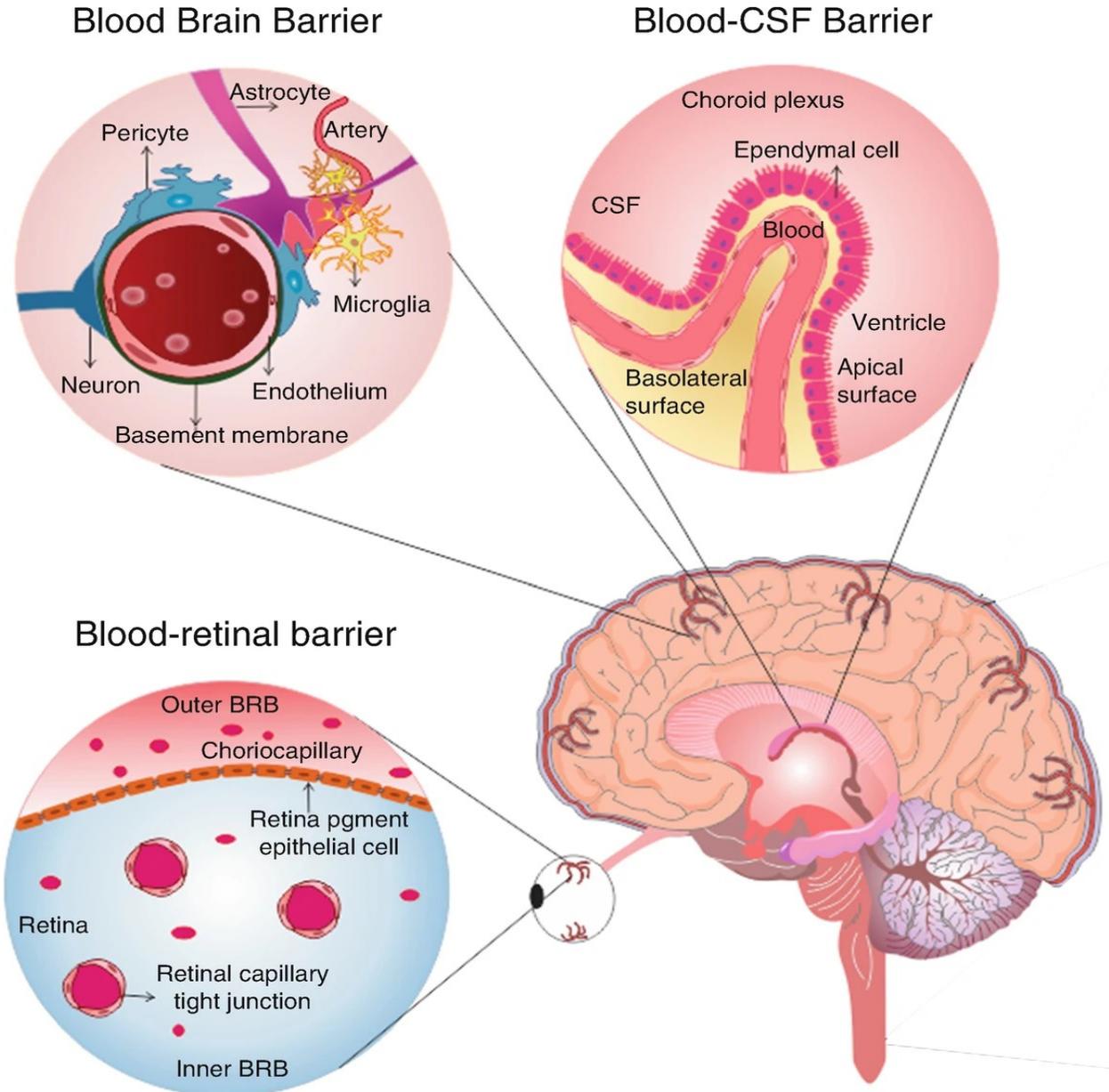


Neural Barriers

Protects the brain from toxins and pathogens

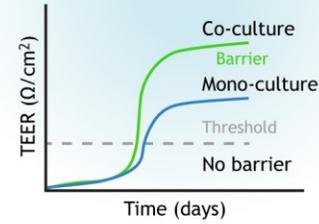
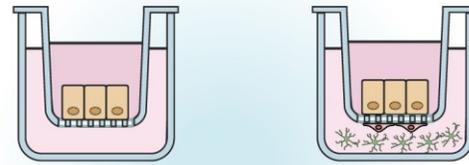
Self-assembling iBRB and traditional oBRB models available

Malarial Retinopathy



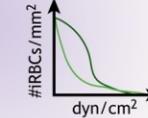
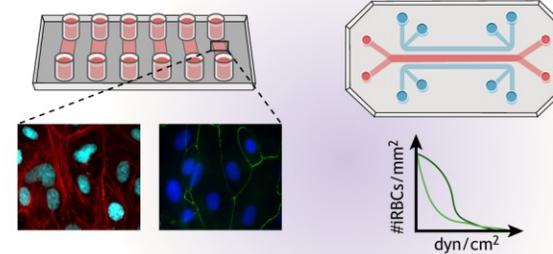
Site of entry to CNS for pathogens:
Bacterial - *Borrelia sp*, *Neisseria meningitidis*
Parasitic – Trypanosomiasis, Malaria
Viral – Echovirus 30

A Mono-culture and co-culture chambers

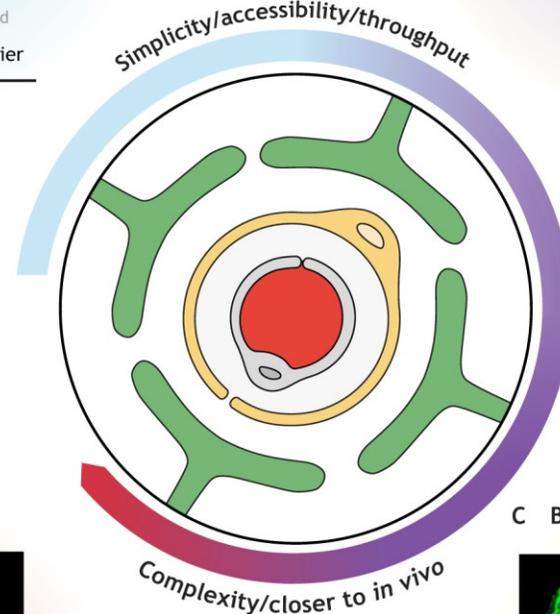


- Barrier integrity via TEER/solute transport in response to iRBCs
- Antibody functionality
- Transmigration of iRBCs/leukocytes on activated barriers

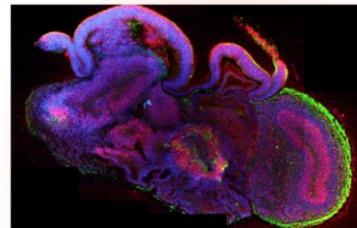
B 2D microfluidic chips, 3D printing and tissue engineering



- Barrier integrity in response to iRBCs
- iRBC adhesion/inhibition under shear stress
- Antibody functionality
- Screening adjunctive treatments

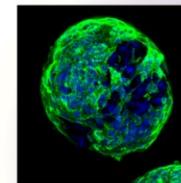


D Cerebral organoids

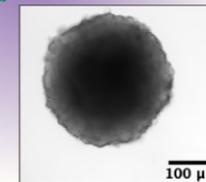


- Neurodevelopment in response to iRBC products
- Toxicity

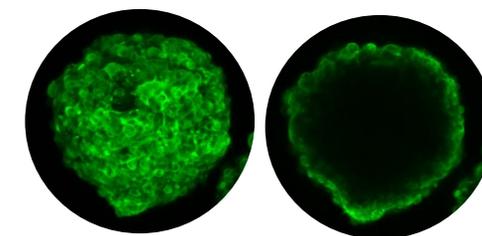
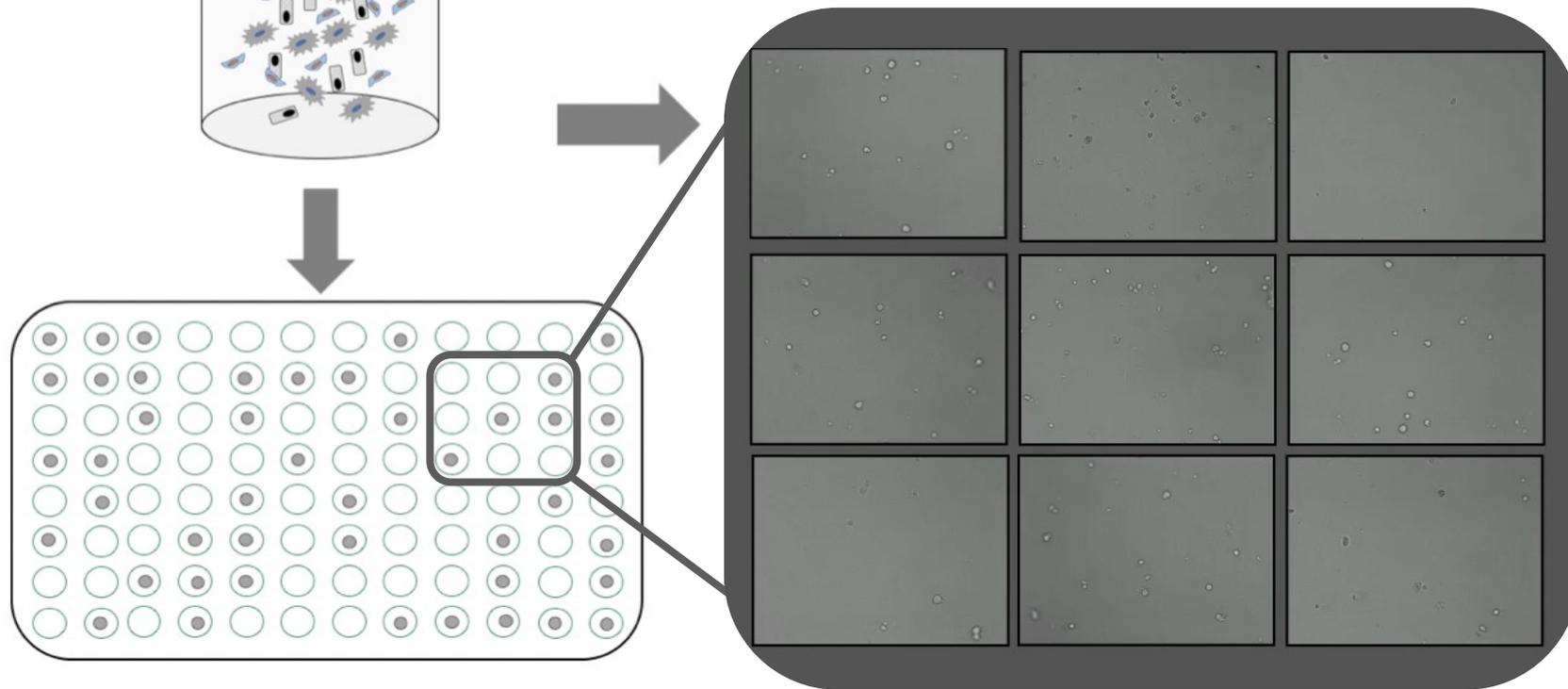
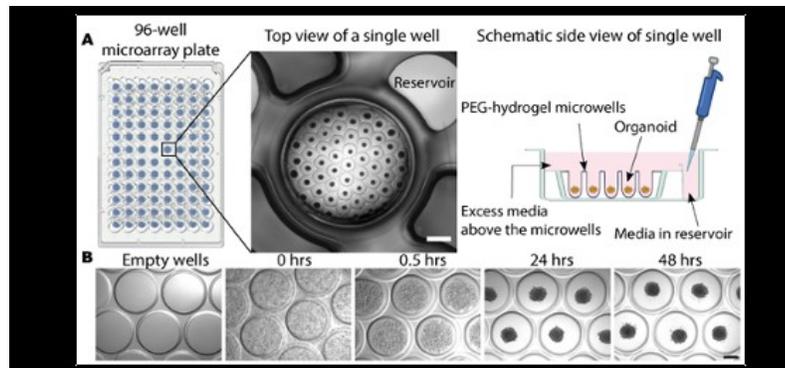
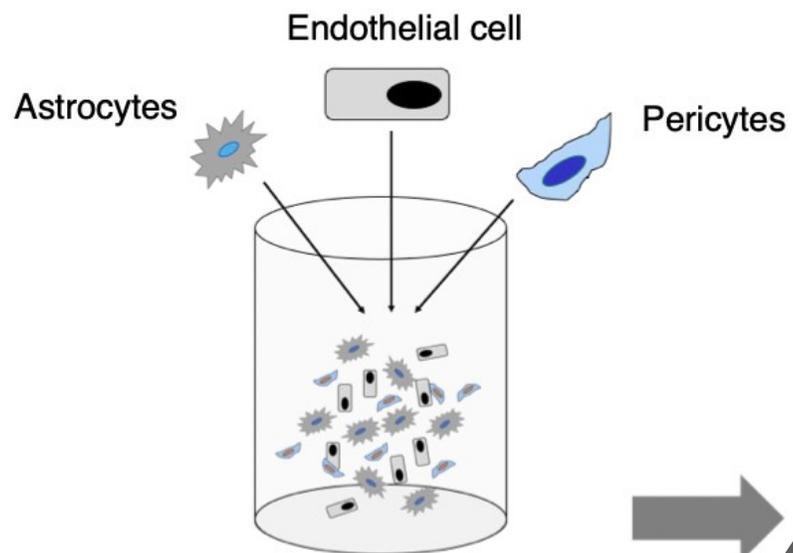
C Blood-brain-barrier organoids



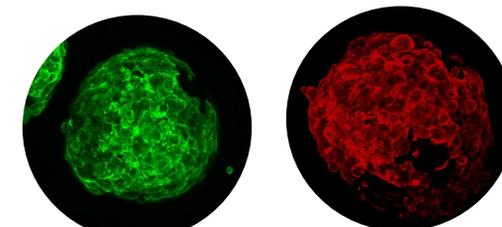
- 'Inside-out' BBB
- Barrier integrity in response to iRBC
- iRBC adhesion/inhibition
- Drug screen to reverse swelling
- Transmigration



Self-assembling Blood-Brain Barrier

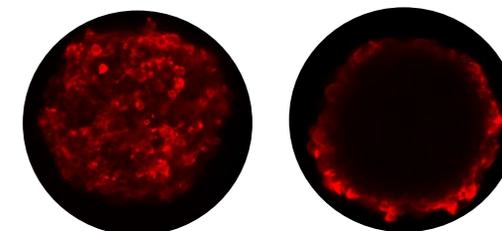


ZO-1



ICAM-1

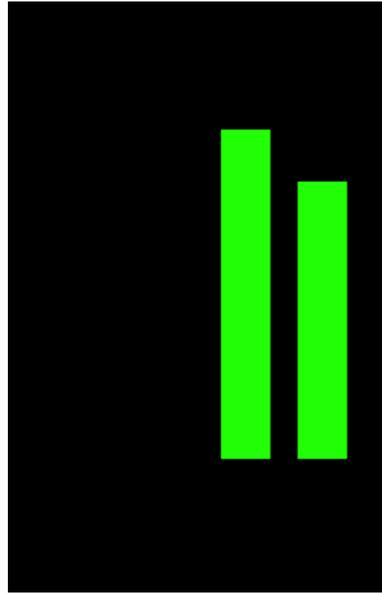
PECAM-1



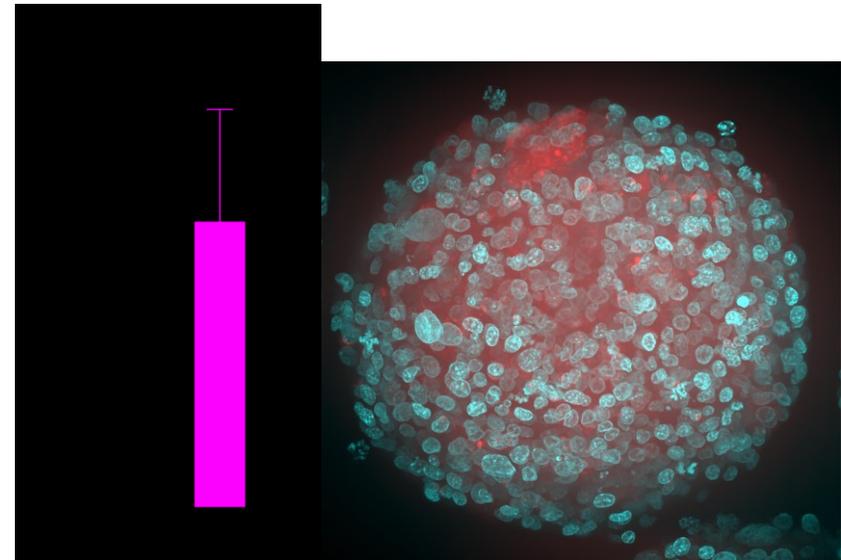
Glycocalyx

Origin of BBB-organoids

Testing if drugs can cross the BBB



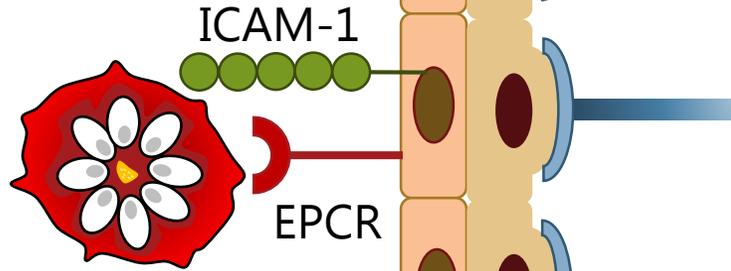
Investigation of fluorescence guided surgery probes



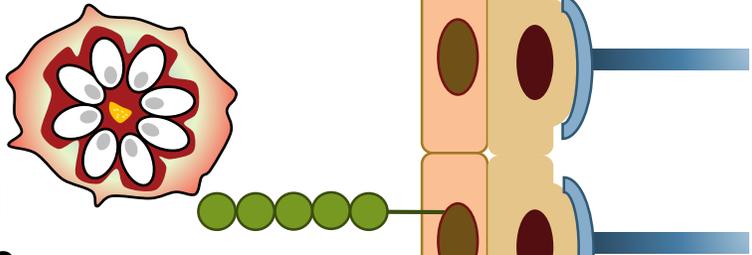
Neuroinvasive pathogens:

Cerebral Malaria

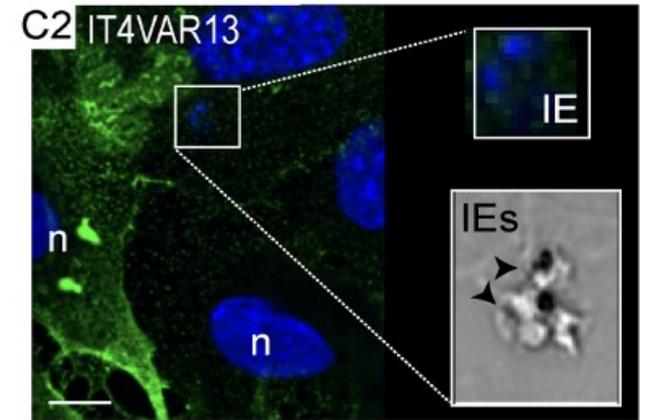
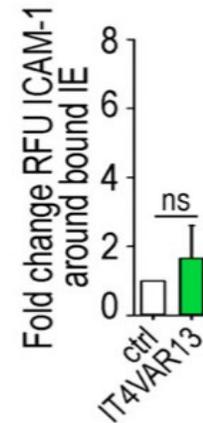
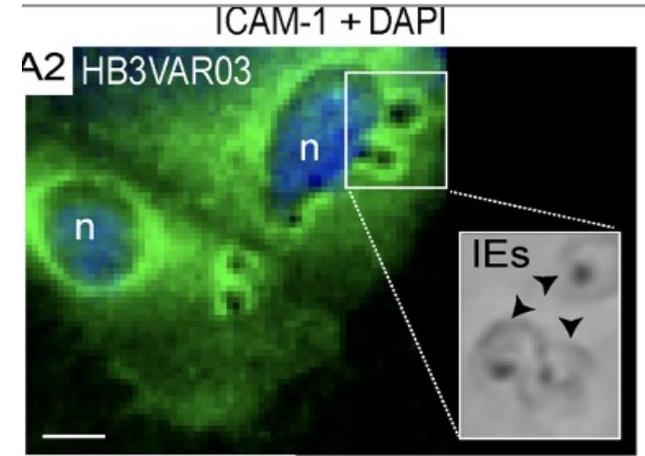
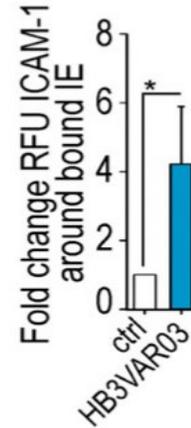
IE group A^{CM}:
CM associated IEs



IE^{non-CM} :
uncomplicated and non-cerebral severe malaria associated IEs



Brain



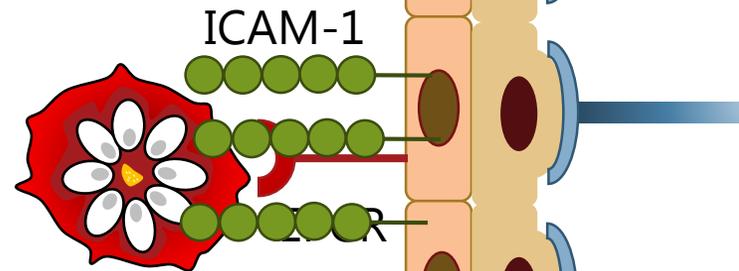
Blood

Brain

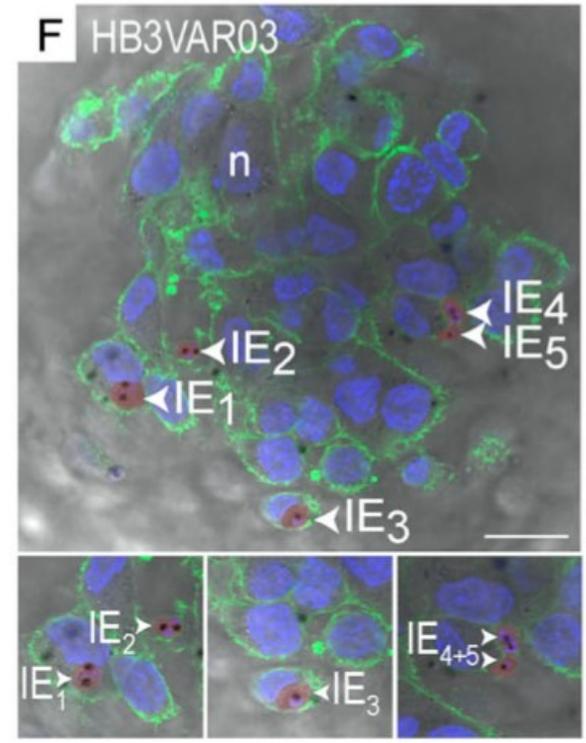
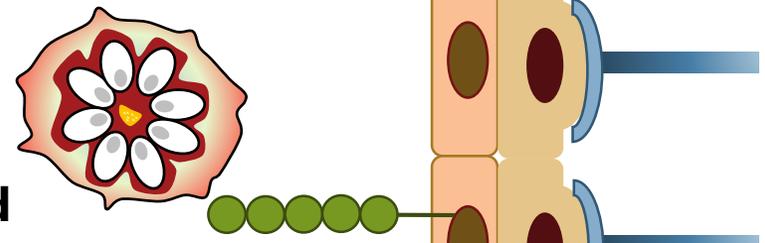
Neuroinvasive pathogens:

Cerebral Malaria

**IE group A^{CM}:
CM associated IEs**



**IE^{non-CM} :
uncomplicated and
non-cerebral severe
malaria associated IEs**



Slice 44 of 60 – 19.8µm

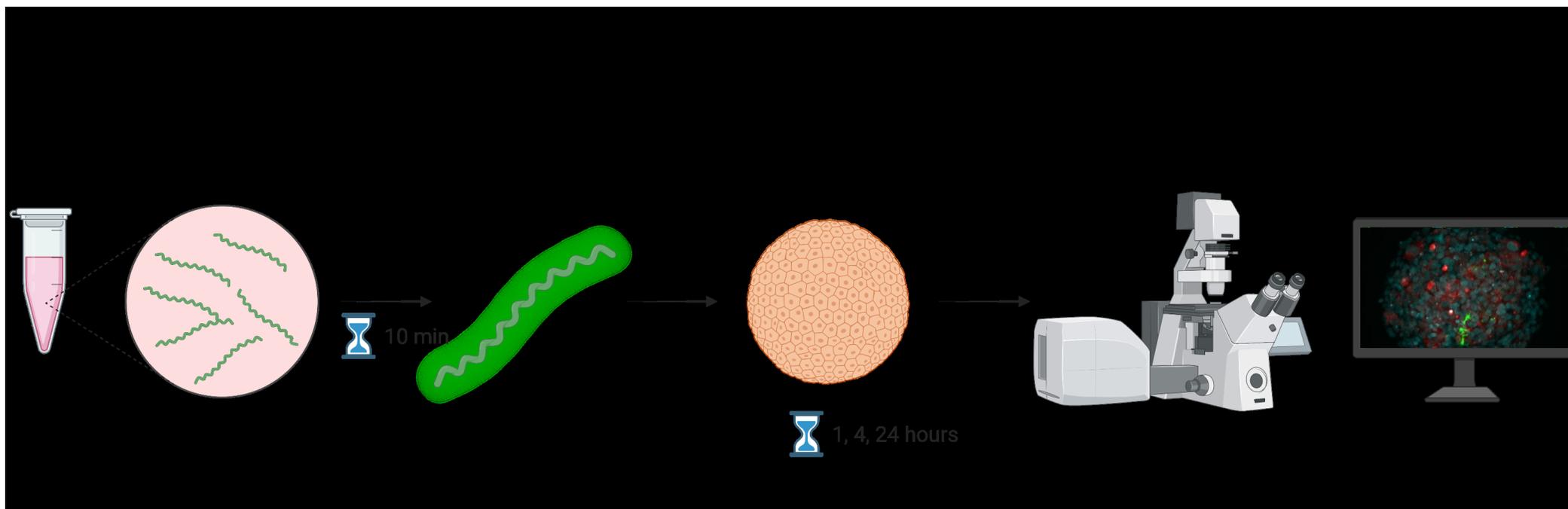
BBB-organoids and Lyme neuroborreliosis

Neuroinvasive

Borrelia garinii – lab/reference strain
Borrelia garinii – patient isolates

Non-neuroinvasive

Borrelia burgdorferi s.s.
Borrelia afzelii
(patient isolates)



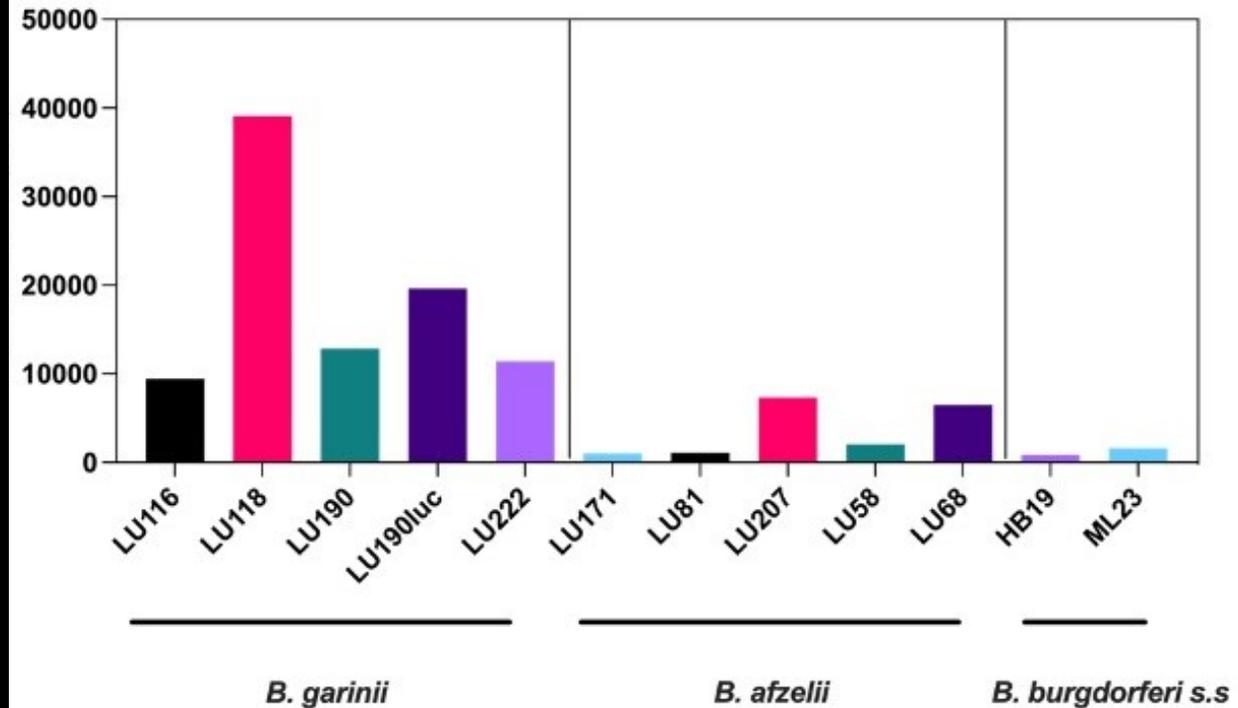
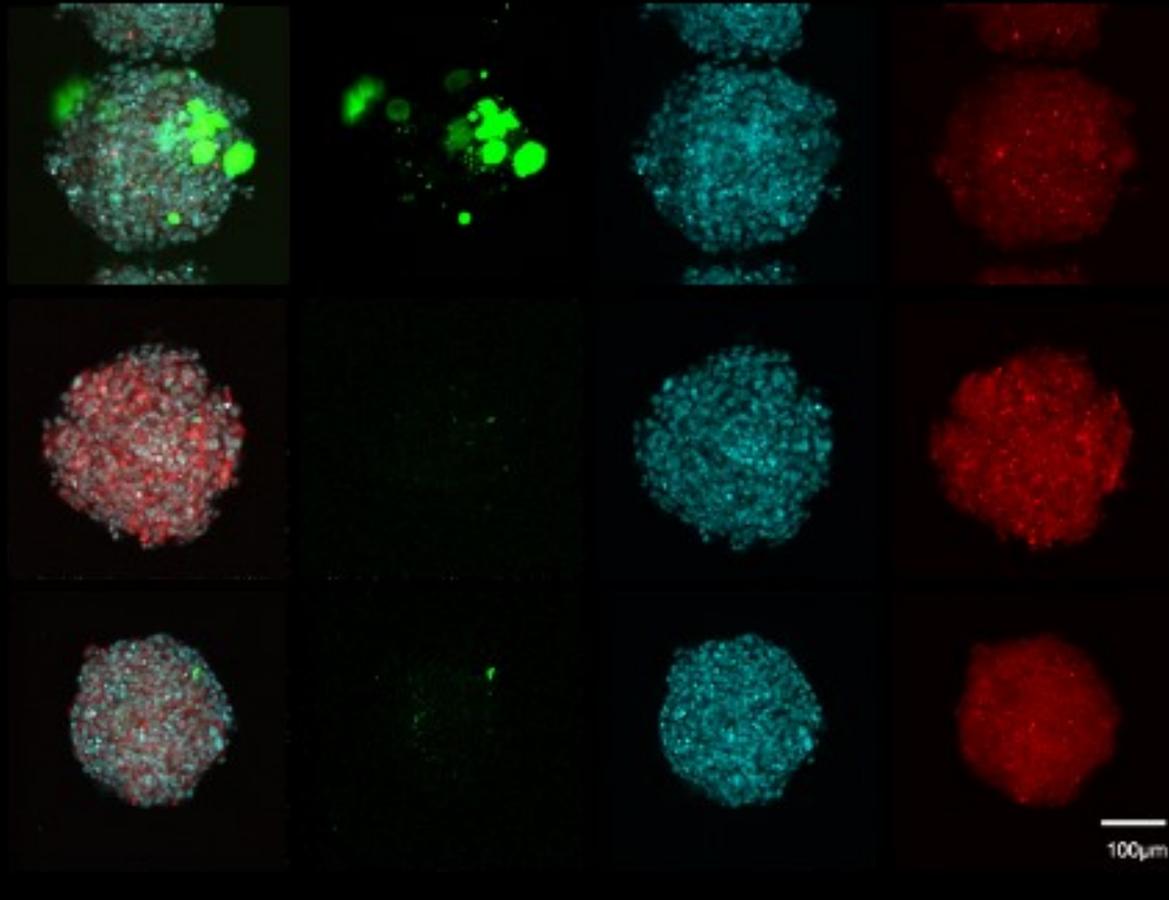
Denmark



Sweden



B. garinii sp. preferentially invade organoids



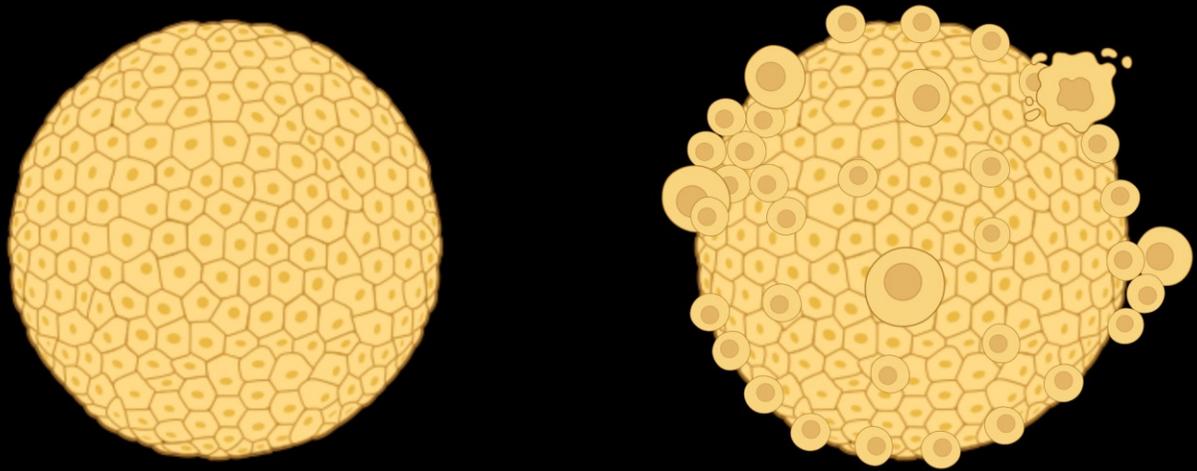
Morphology

Shape Descriptors (previously **Circularity**) - Calculate and display the following shape descriptors

Circ. (circularity) - value approaches 1 for very small

Round (roundness)

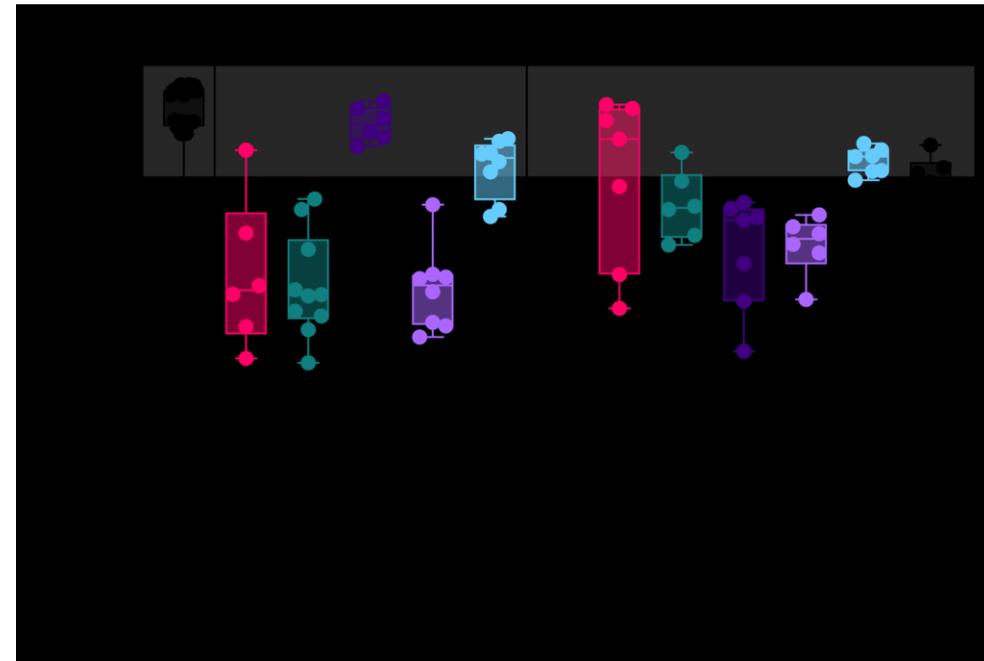
value



Morphology comparison – round and circularity



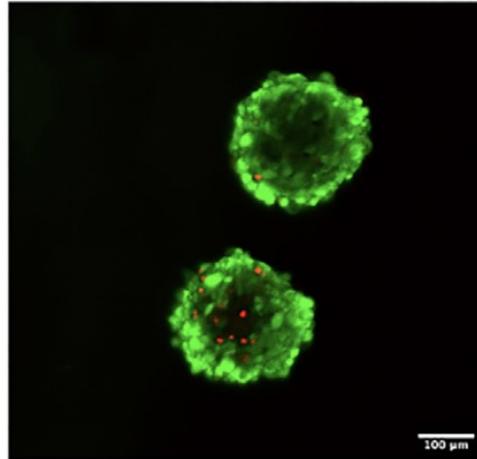
No variation in “round” values =
organoids are consistent in overall shape



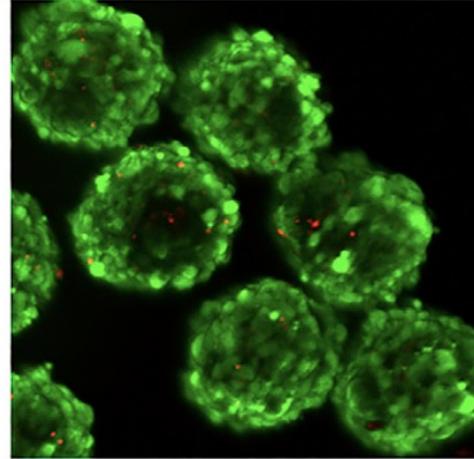
Variation in “circularity” values =
organoids are inconsistent between
groups due to lack of cohesion/perimeter
values more variable compared to
radius/diameter.

Organoid Integrity: loss of tight-junctions does not affect viability

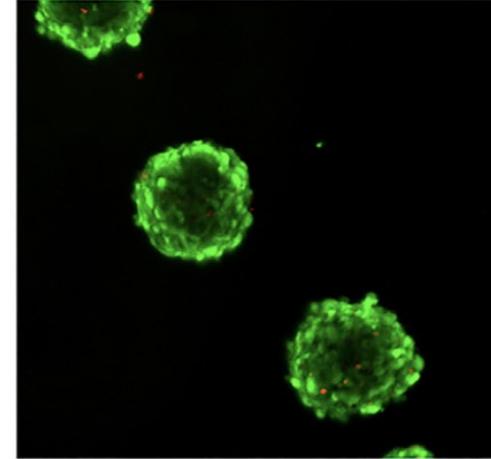
A Mock Treated



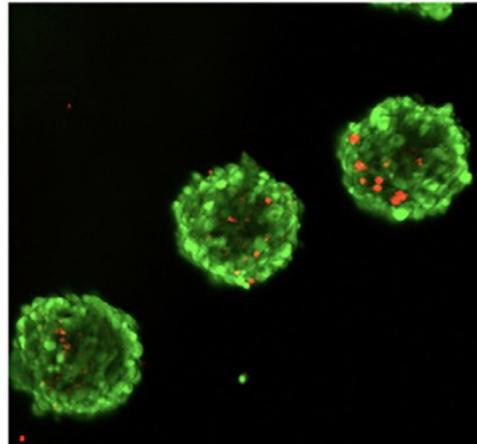
B *B. Burgdorferi* s.s (ML23)



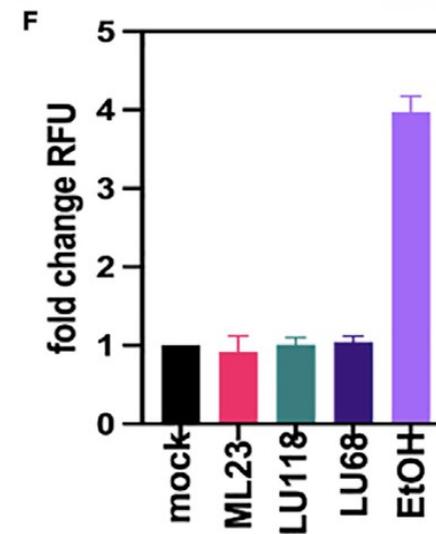
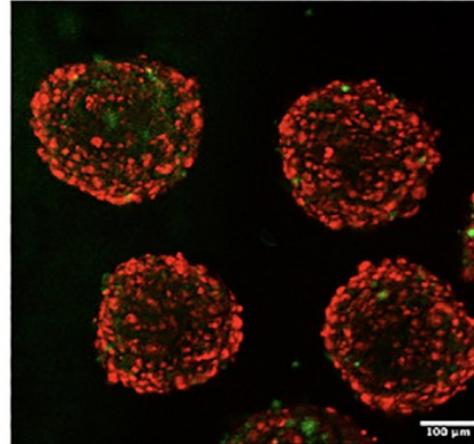
C *B. afzelii* (LU68)



D *B. garinii* (LU118)



E 70% EtOH (20 mins)

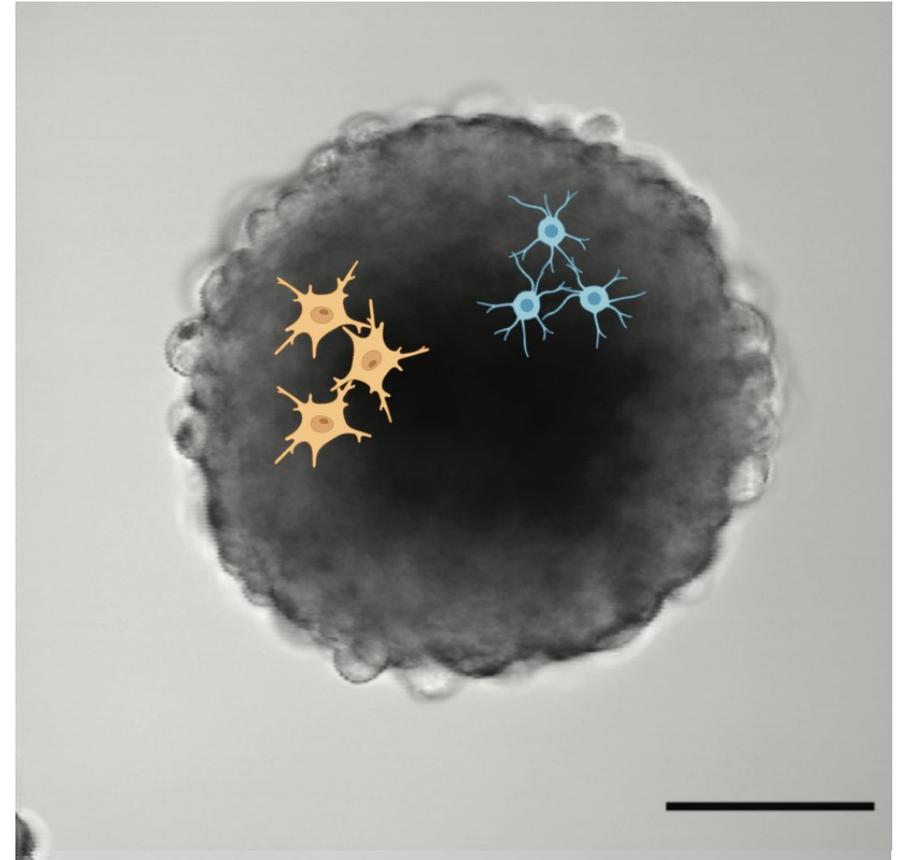


Future directions...

Development of immuno-competent BBB-organoids

- addition of microglia and oligodendrocytes

Measure the immune responses generated by immuno-competent organoids



Future directions...

Blood-CSF barrier:

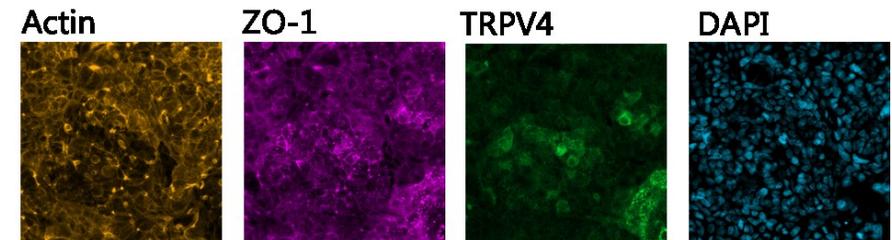
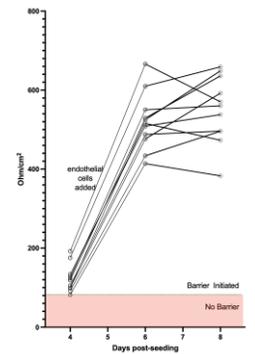
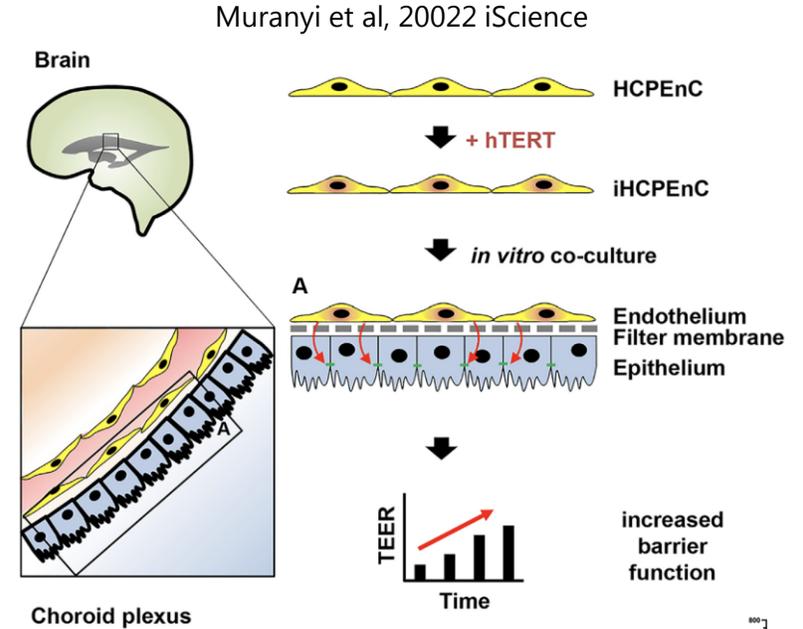
- modelling the choroid plexus

Current organoid model lacks endothelial cells

- co-culture model

Investigate impact of immune cells and malaria

Does borrelia use BCSFB to gain entry to CNS?



Summary

Easily produced in vitro: >95% success rate in culture

Support the adhesion and invasion of *Plasmodium falciparum* infected red cells

Support the invasion of Lyme neuroborreliosis genospecies

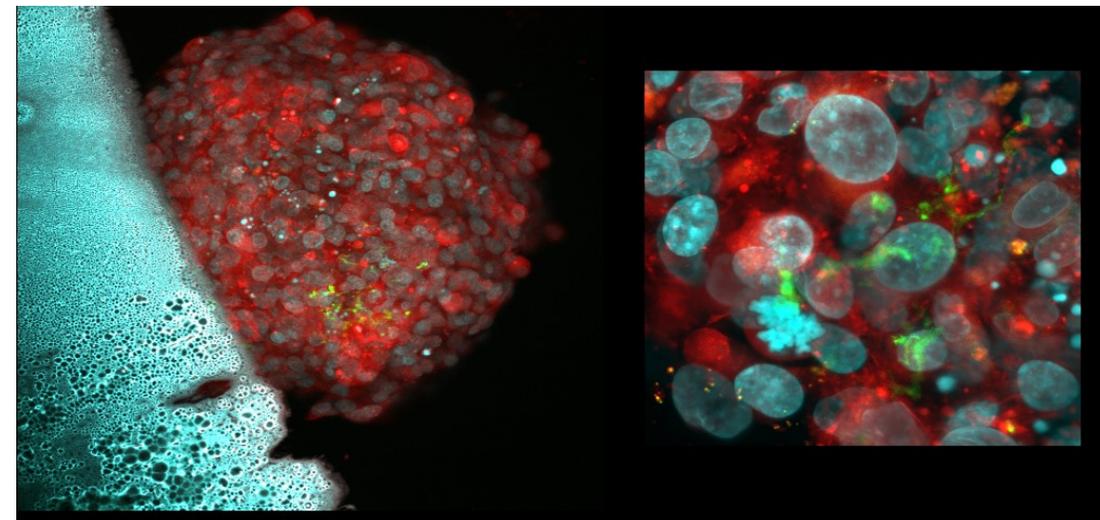
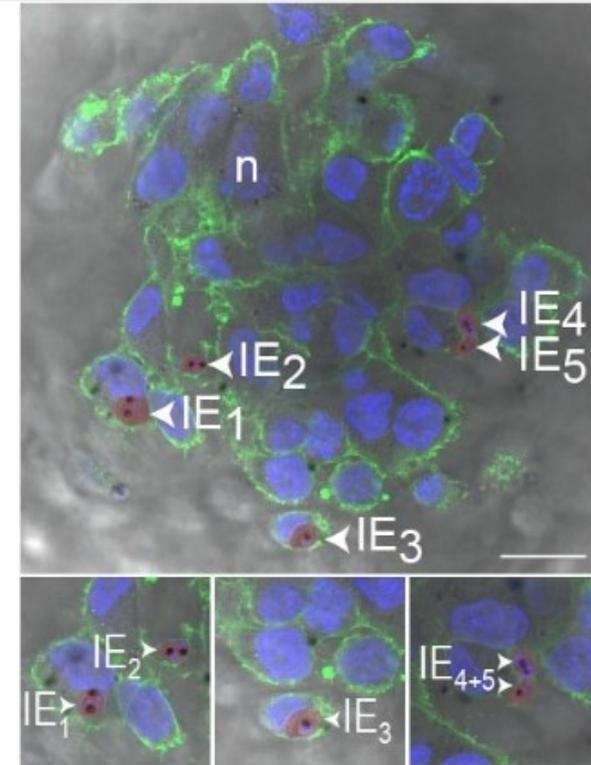
Provide a means to measure gross morphological changes to organoids in response to pathogens

Assess the impact of pathogens on barrier dysfunction

Excellent platform to examine the effects of other neuroinvasive bacterial, parasitic, or viral infections

Next-gen organoids – immuno-competent

Approx. 20µm within organoid. IEC^{CM} false coloured red to aid visualisation.



TARGETS

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 Katrine W Zeeberg
 Mette Ulla Madsen
 Amalie Bisholm

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 Gertrude Ecklu-Mensah
 Katrine J Hansen
 Jonas Rudbæk
 Nanna Dalgaard

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Helene Mens RH (Borrelia)
Daniel Farhoul-Jepsen RH (Borrelia)
Peter Østrup Jensen RH (Borrelia)
Andreas Kjær UCPH (Glioblastoma)
Trine L Toft UCPH (Choroid Plexus)
Thomas Bjarnsholt UCPH (Borrelia)
Kasper N Kragh (Borrelia)

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Tara Walker (Neurodegeneration)
 Queensland Brain Institute

Germany

Horst Schroten (Neural Barriers)
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 Mannheim University Hospital

Japan

Tomomi Furihata (BBB)
 Tokyo University
Hiroshi Ishikawa (Neural Barriers)
 Nippon Dental University

Sweden

Per-Erik Lindgren (Borrelia)
Anna Hennignsson (Borrelia)
Sofie Haglund (Borrelia)
 University of Linköping

UK

Sam Wassmer (malaria)
 LSHTM

USA

Sean Lawler (Glioblastoma/Drug transport)
 Brown University, RI



The Lundbeck
 Foundation Investigator
 Network

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Borrelia Research part of:

