



Webinar

***Cerebral Open Flow
Microperfusion (cOFM)
for In-Vivo Cerebral
Fluid Sampling -
Comparison of cOFM
and Microdialysis***

October 14th, 2021

Who We Are

Today's Speaker



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Who We Are

Boutique CRO for research projects & drug development programs



We support clinical and preclinical activities by providing:

- (Pre)clinical PK/PD/BE studies at the target tissue level
- In-vitro release testing (IVRT)
- Customized bioanalyses (PK, PD) – GLP certified lab
- Metabolomics
- Data management
- Biostatistics
- Medical writing

Who We Are

Open Flow Microperfusion (cOFM)



Dermal Tissue



Adipose Tissue



Brain Tissue



EX-VIVO

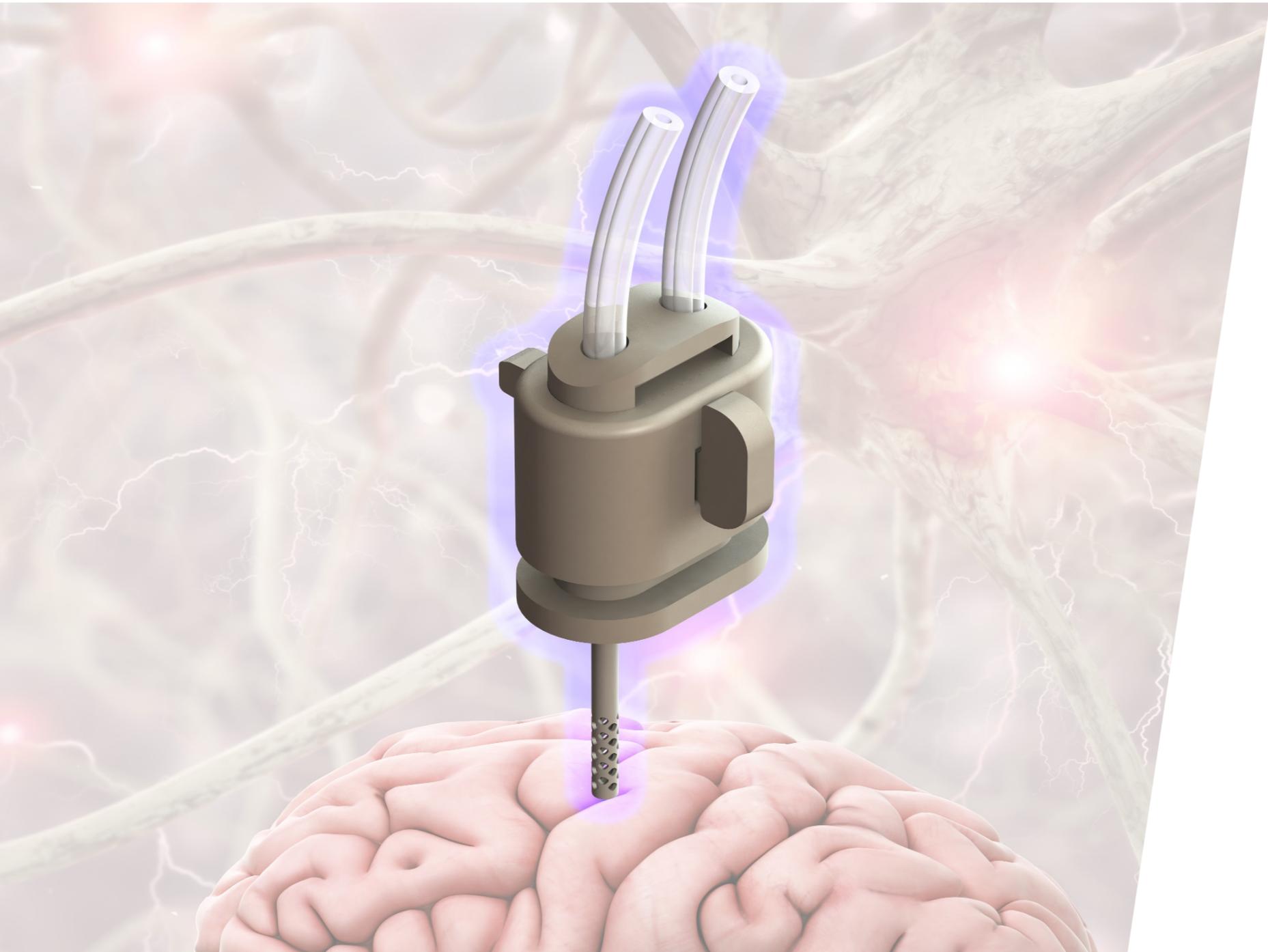


PRE-CLINICAL



CLINICAL

- ✓ provides unfiltered interstitial fluid
- ✓ PK/PD at target tissue
- ✓ CE-certified for clinical use (aOFM/dOFM)

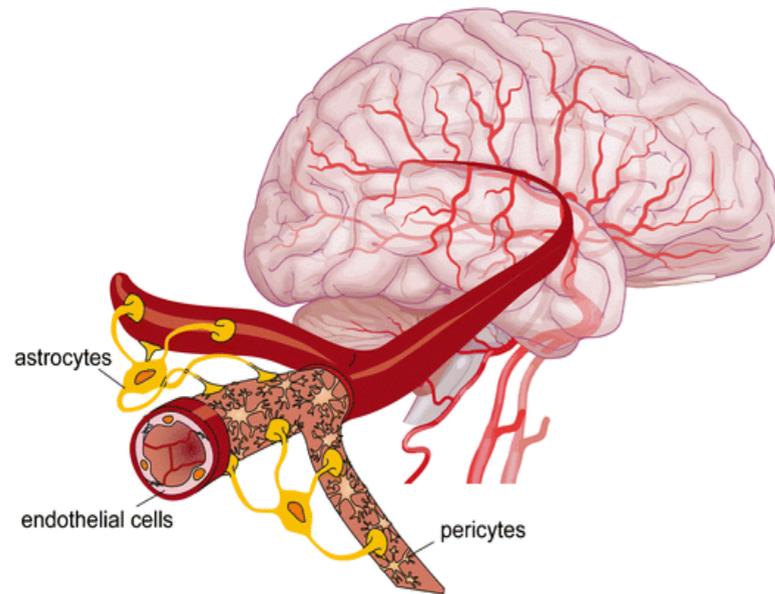


Biomedical Tissue Monitoring in the Brain

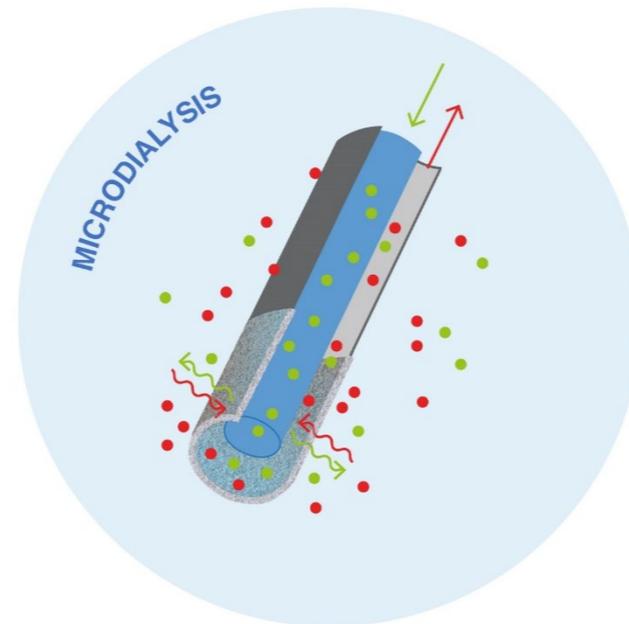
6  Biomedical Tissue Monitoring

How to Measure Substances in the Brain In-Vivo?

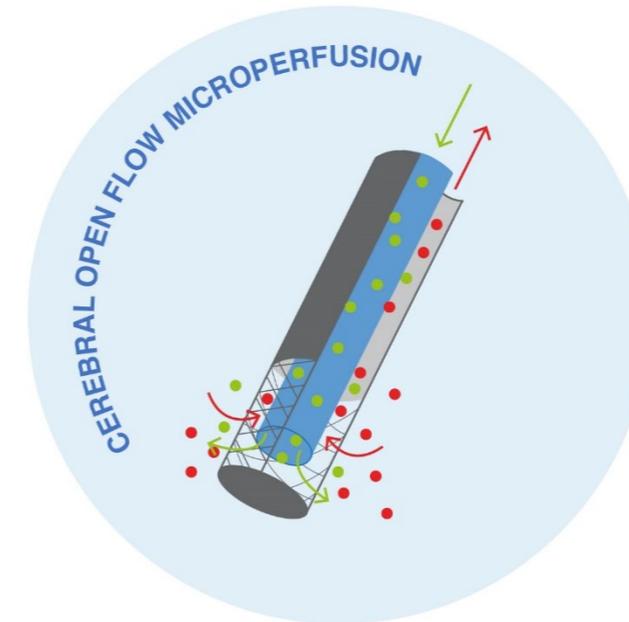
Blood-Brain Barrier (BBB)



Microdialysis



cOFM



Features:

- no membrane
- no size exclusion
- unfiltered cISF
- intact BBB
- long sampling times

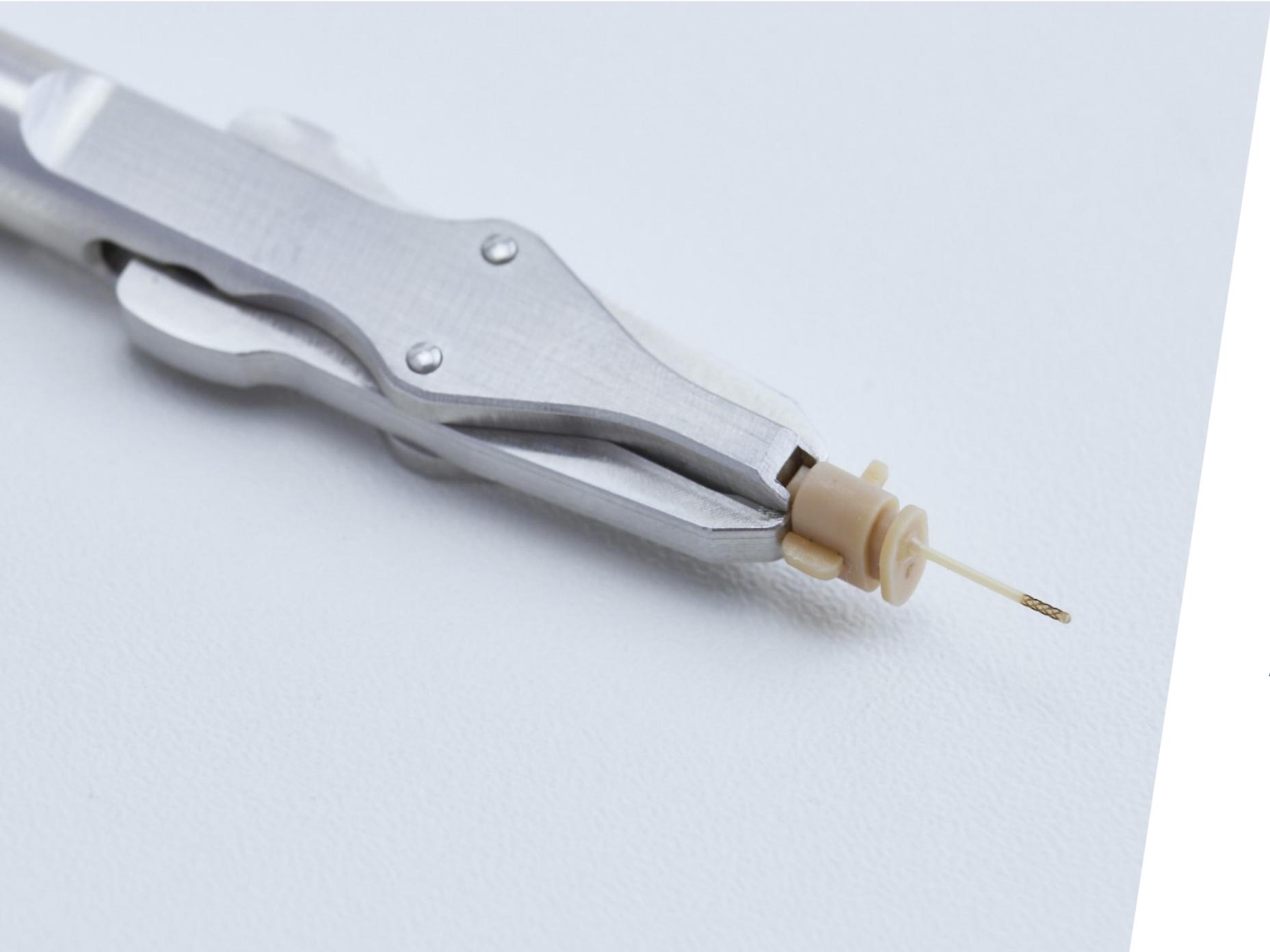
Credit: https://www.researchgate.net/figure/Brain-vasculature-separates-the-circulating-blood-from-the-CNS-tissue-The-vessels-are_fig1_320026611

Credit: JOANNEUM RESEARCH

Question

I am working with ...

- MD
- a/dOFM
- cOFM
- others

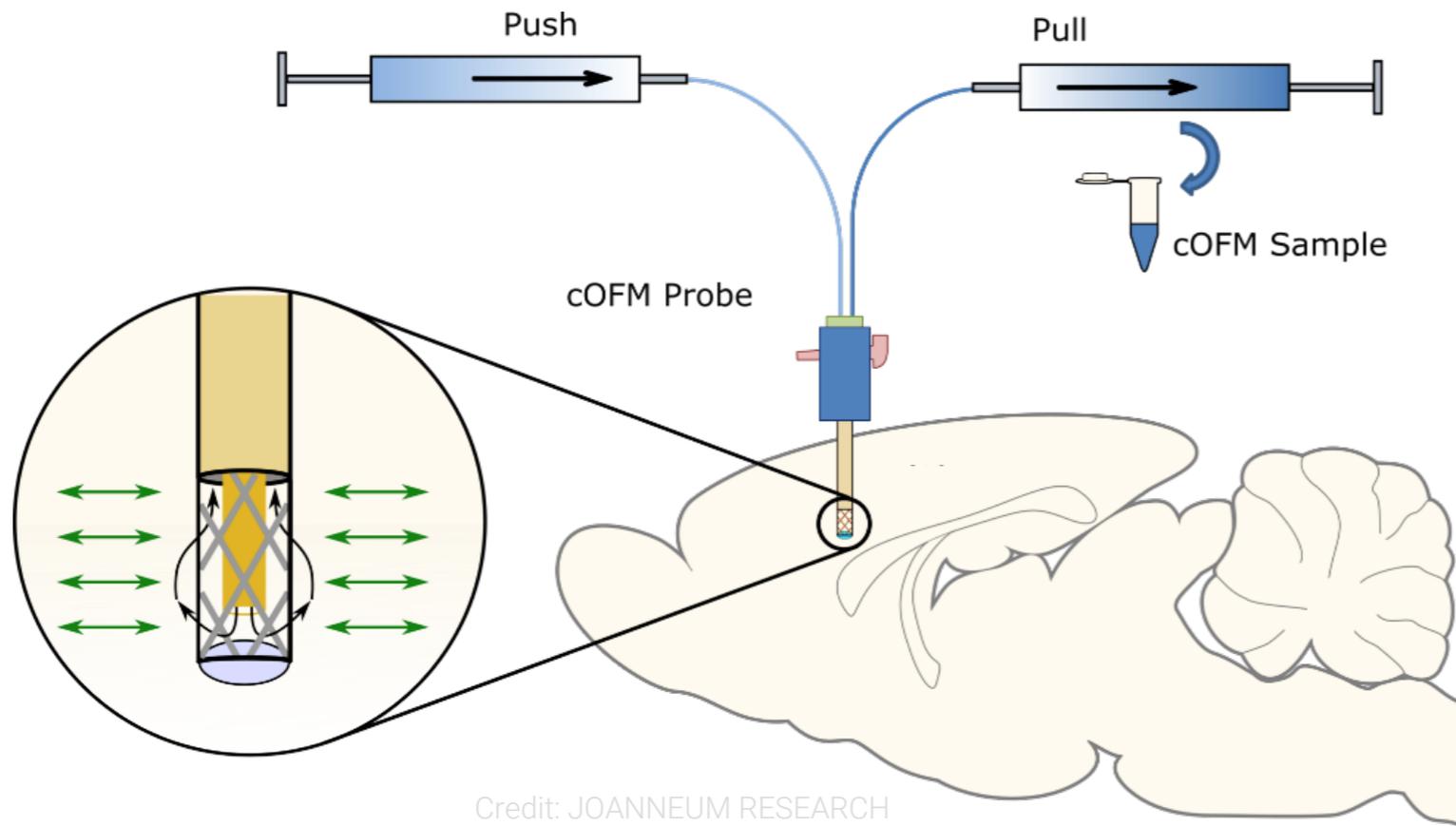


 eOFM

***Cerebral
Open Flow
Microperfusion***

cOFM Cerebral Open Flow Microperfusion

Concept

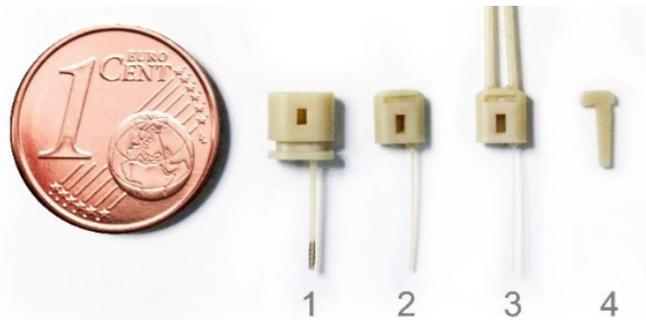


Credit: JOANNEUM RESEARCH

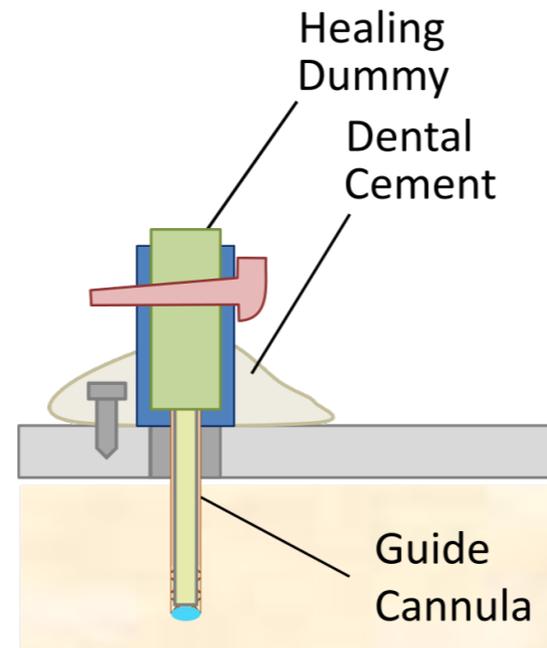
cOFM is a unique tool to investigate pharmacokinetics (PK) and pharmacodynamics (PD) in the brain with an intact BBB.

COFM Cerebral Open Flow Microperfusion

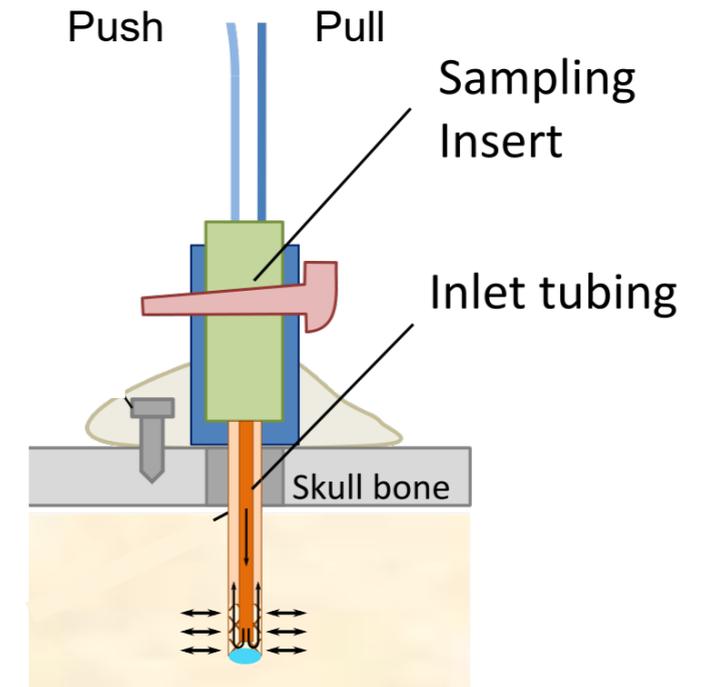
Implantation and Sampling



- 1 - Guide \varnothing 0.5 mm
- 2 - Healing Dummy
- 3 - Sampling Insert
- 4 - Locking Wedge



14 days

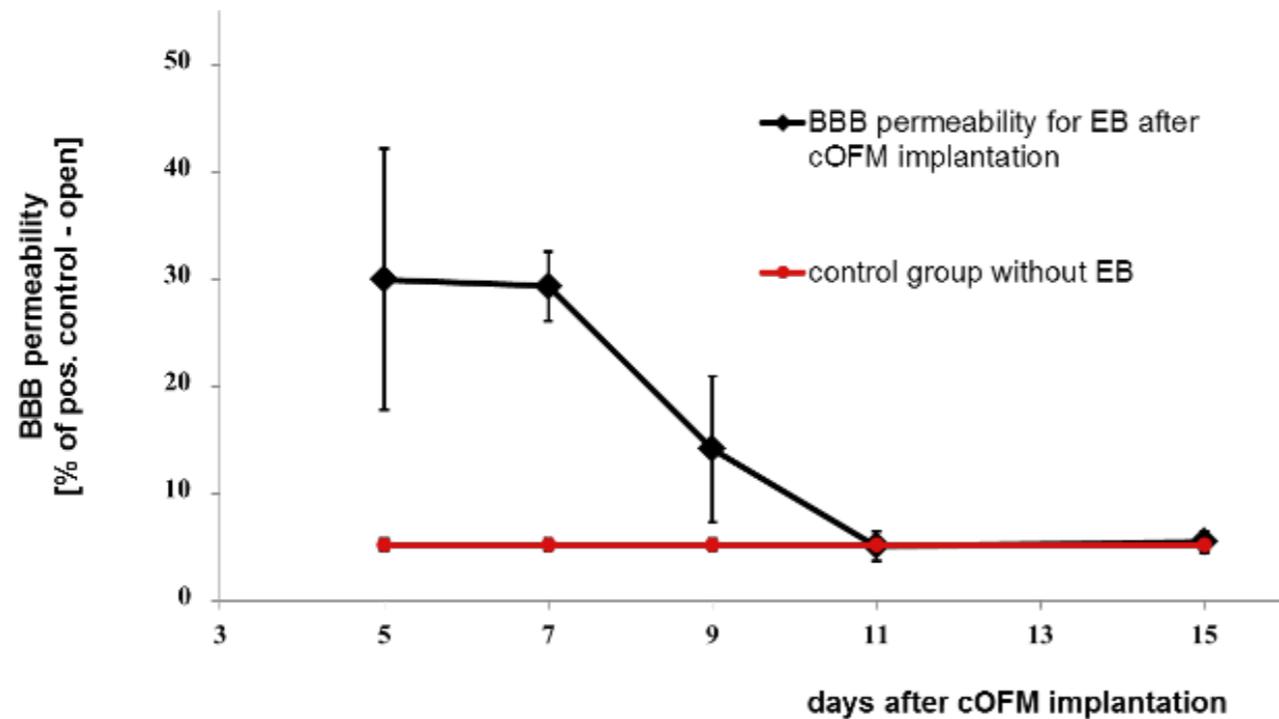


Implantation and sampling are 14 days apart
 ⇒ **intact Blood Brain Barrier**

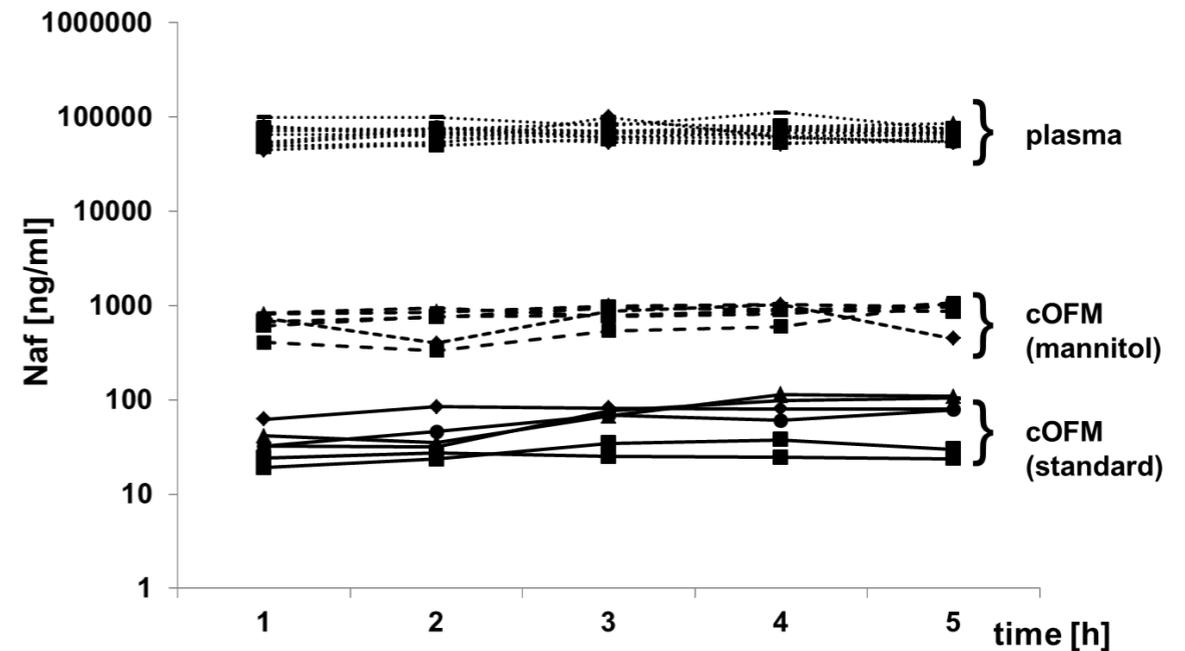
cOFM Cerebral Open Flow Microperfusion

Intact Blood Brain Barrier

Evans Blue BBB permeability

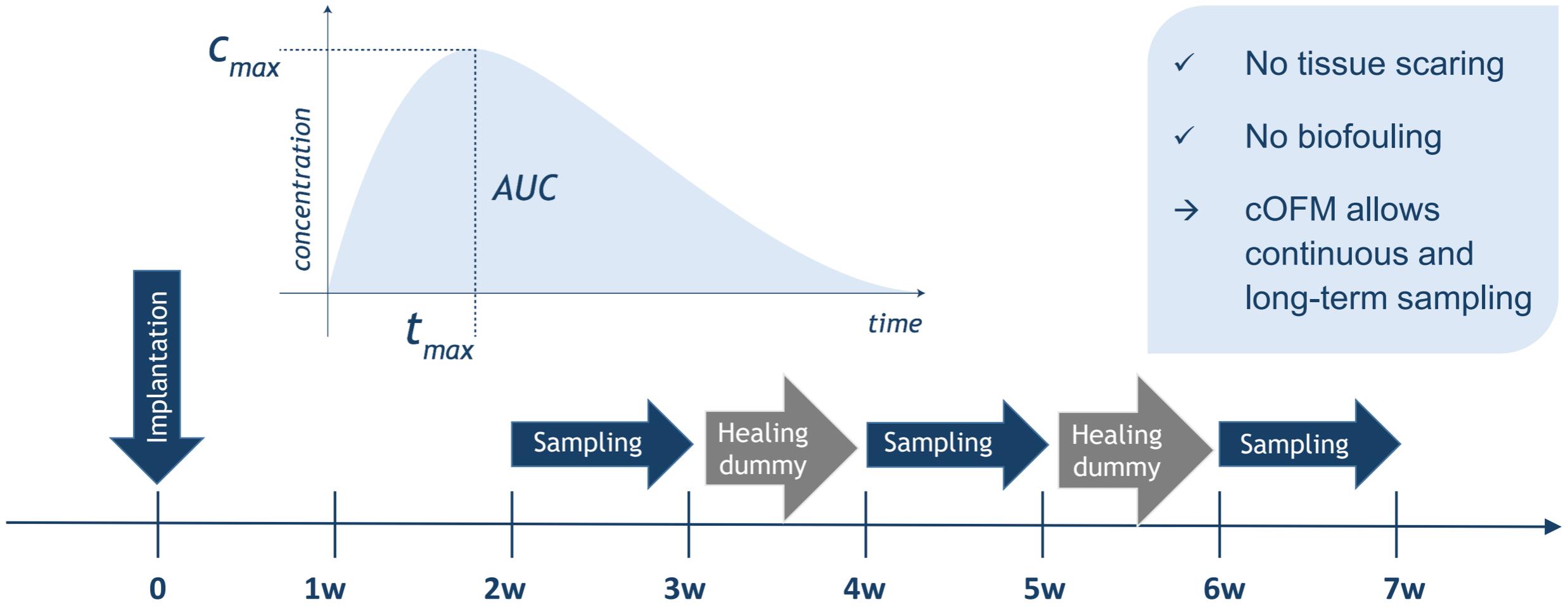


BBB marker – Sodium fluorescein (Naf)



cOFM Cerebral Open Flow Microperfusion

Time-Resolved Substance Concentration Monitoring

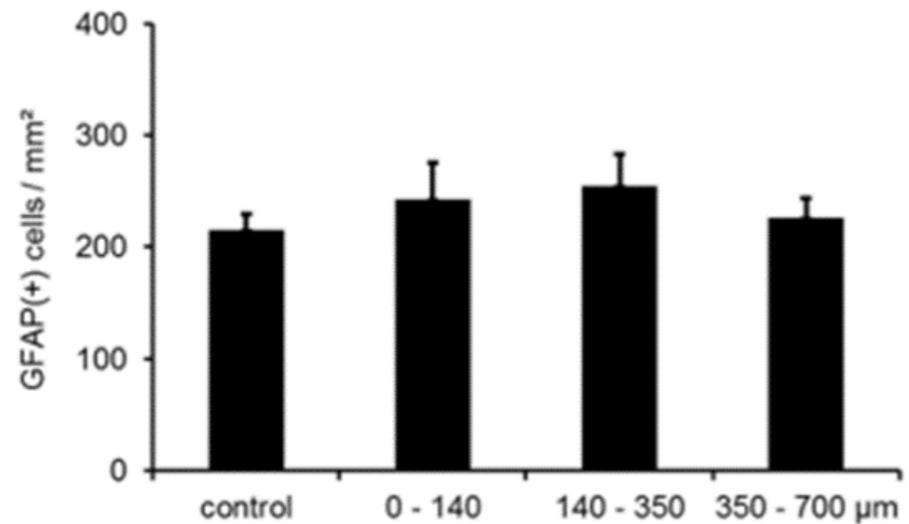


- ✓ No tissue scaring
- ✓ No biofouling
- cOFM allows continuous and long-term sampling

cOFM Cerebral Open Flow Microperfusion

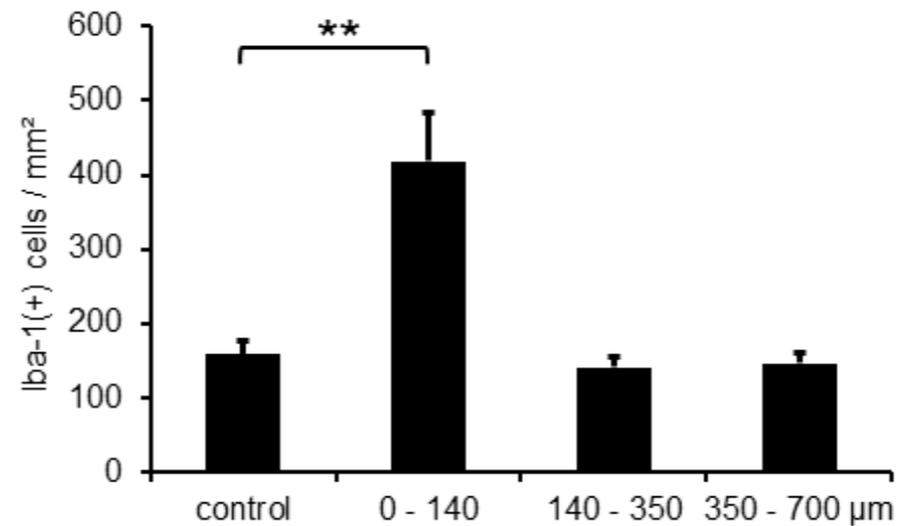
Minimal Tissue Reaction

Quantification of Astrocytes



Distance to cOFM probe

Quantification of Macrophages



Distance to cOFM probe

- ✓ cOFM probe material elicits minimal tissue reaction.
- ✓ No glial scar
- ✓ 15 days and 30 days after cOFM implantation



OFM Cerebral Open Flow Microperfusion

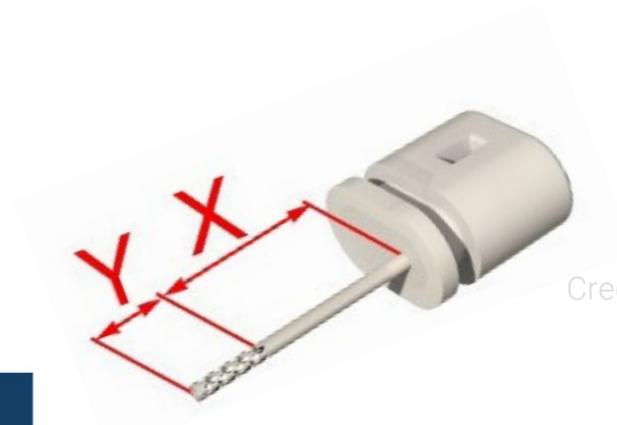
Different Brain Regions

ISF sampling in

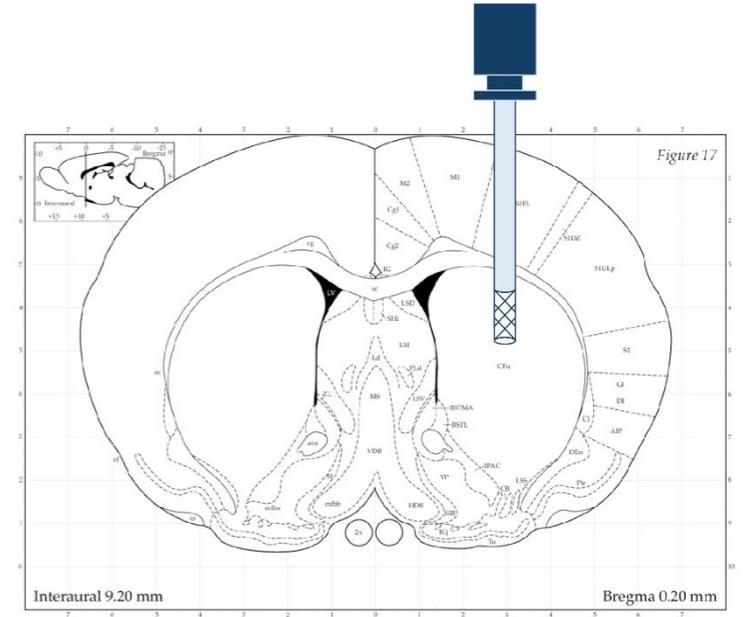
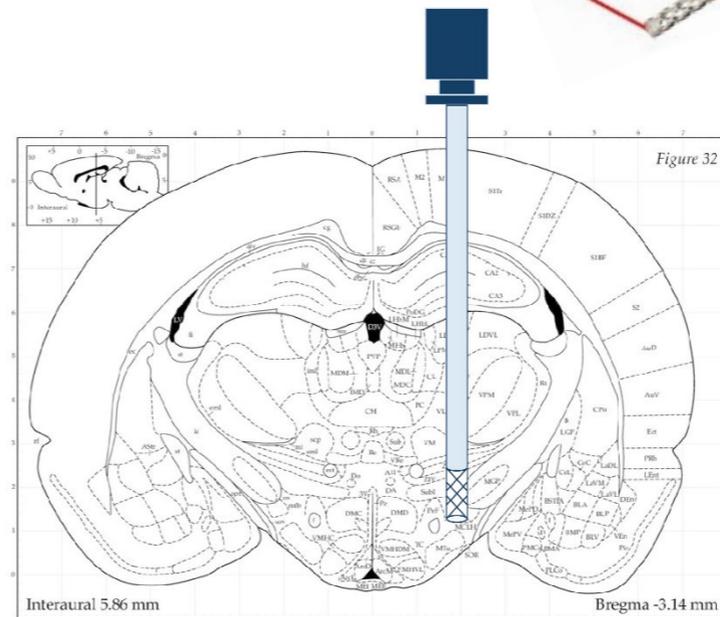
- striatum
- cortex
- hippocampus
- hypothalamus
- ..and others

CSF sampling in

- lateral ventricle
- third ventricle



Credit: JOANNEUM RESEARCH



COFM Cerebral Open Flow Microperfusion

Monitoring of Substances without Limitation

- **Lipophilic substances**
 - Amitriptyline (logP: 4.9), fluoxetine (logP: 4.6), ...
- **Big substances**
 - Antibodies (trastuzumab, anti-BACE1, ...)
 - Proteins (amyloid β , tau, albumin, IGGs, ...)
 - Nanoparticles (doxorubicin, liposomes, ...)
- **Hormones**
 - Leptin (16 kDa), GLP-1 (3297 Da)
- **Biomarkers**
 - Cytokines, eicosanoids, growth factors, glucose, ...



Question

Proteins or compounds that I am interested in measuring are:

- a) Neurotransmitters
- b) Neuropeptides
- c) Hormones
- d) Antibodies
- e) Drugs
- f) Nanoparticles
- g) Proteins
- h) Growth factors
- h) Cytokines
- J) others

Cerebral Open Flow Microperfusion

Applications

BBB

Monitoring of BBB function and permeability changes

Pharmacokinetics

Monitoring of substance transport across the intact BBB & time-resolved concentration profile in the brain

Pharmacodynamics

Monitoring of biomarkers in the brain

Established:

- Rodents (mice, rats)

Ongoing Development:

- Pigs
- Non-human primates
- Humans

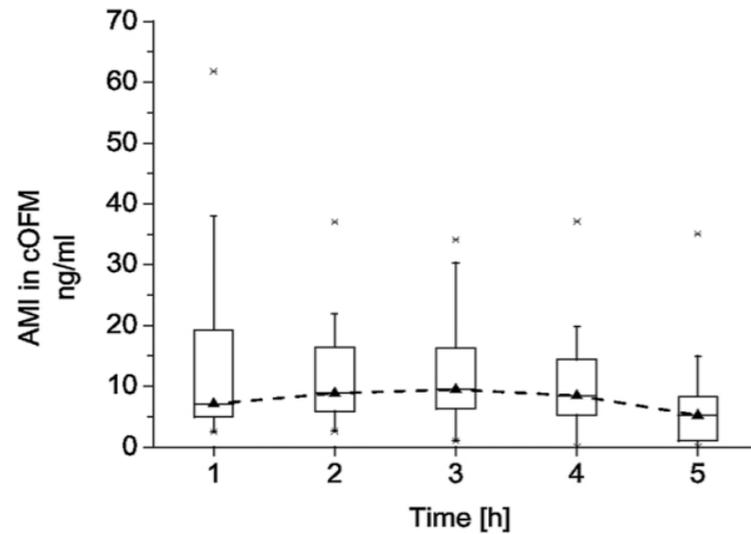


Case Study

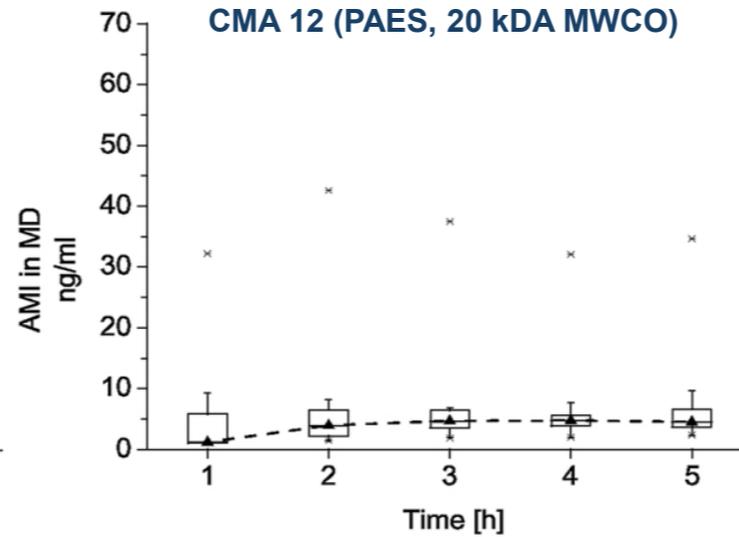
Amitriptyline Sampling with cOFM and Microdialysis

Small (277 Da); lipophilic (logP 4.92); highly protein bound (95%); dosing: 25 mg/kg intraperitoneal

cOFM



Microdialysis



Analyte	Sampling Method	AUC (ng*h/mL)
AMI	cOFM	33.5
	MD	16.5
HYA	cOFM	9.4
	MD	n.a.
ANO	cOFM	13.9
	MD	n.a.
NOR	cOFM	n.a.
	MD	n.a.



Altendorfer-Kroath T, Schimek D, Eberl A, Rauter G, Ratzner M, Raml R, et al. Comparison of cerebral Open Flow Microperfusion and Microdialysis when sampling small lipophilic and small hydrophilic substances. J Neurosci Methods 2019;311:394–401. <https://doi.org/10.1016/j.jneumeth.2018.09.024>.



Other Applications

■ **Neurodegenerative Diseases**

Collecting antibodies and large molecule biomarkers in mouse interstitial brain fluid: a comparison of microdialysis and cerebral open flow microperfusion. Le Priault et al. (2021), mAbs, 13:1, 1918819, DOI: 10.1080/19420862.2021.1918819

■ **Obesity/Diabetes**

Time-resolved hypothalamic open flow micro-perfusion reveals normal leptin transport across the blood–brain barrier in leptin resistant mice, Maximilian Kleinert (2018). Molecular Metabolism, Volume 13, Pages 77-82. DOI: 10.1016/j.molmet.2018.04.008

■ **Inflammation/BBB integrity**

Assessment of blood-brain barrier function and the neuroinflammatory response in the rat brain by using cerebral open flow microperfusion (cOFM). Ghosh et al., PLoS One. 2014 May 22;9(5). DOI: 10.1371/journal.pone.0098143.

■ **Nanoparticles**

Enhanced doxorubicin delivery to the brain administered through glutathione PEGylated liposomal doxorubicin (2B3-101) as compared with generic Caelyx,(®) / Doxil (®) -- cerebral open flow microperfusion pilot study. Birngruber et al. (2014) J Pharm Sci. 2014 Jul;103(7):1945-1948. DOI: 10.1002/jps.23994

■ **Glioblastoma (work in progress)**

question

I am interested in learning about

- a) cOFM & Neurodegeneration
- b) cOFM & Obesity/Diabetes
- c) cOFM & Glioblastoma
- d) cOFM & Nanoparticles
- E) cOFM & BBB integrity monitoring
- F) cOFM & Absolut quantification

cOFM Cerebral Open Flow Microperfusion

cOFM service @ JR HEALTH

- cOFM setup is combined with  BASi
Raturn[®] and Culex[®]
- Simultaneous dosing & sampling
(cISF, CSF & blood)
- Awake, freely moving animals



Key Learnings

-  cOFM samples cerebral fluids with an intact blood brain barrier.
-  cOFM is membrane-free and samples all substances from the interstitial fluid independent of size, lipophilicity, or protein-binding.
-  cOFM enables continuous sampling for up to several days and a long implantation period for up to several weeks.
-  cOFM studies provide data for drug development as well as unique insights into brain metabolism and signaling.

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