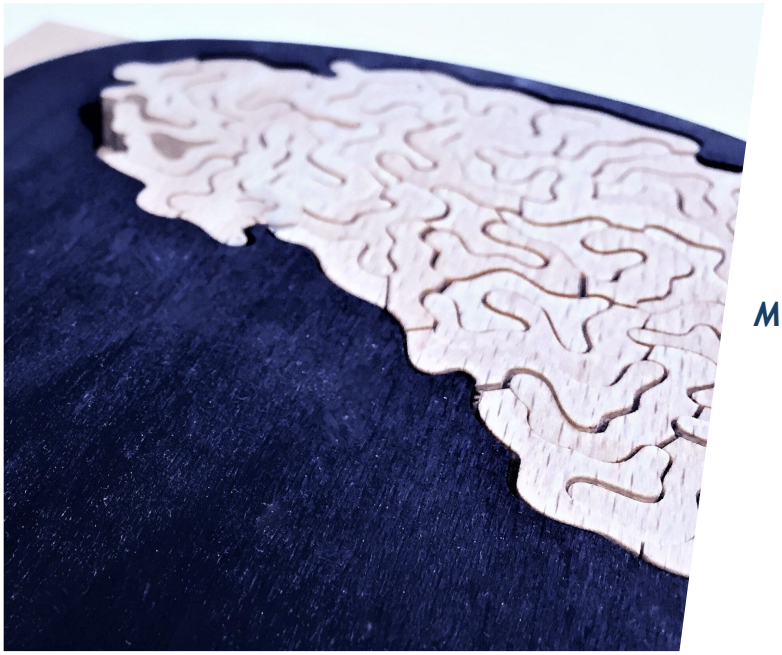

Webinar

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

Joanna Hummer, PhD

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Joanneum Research HEALTH

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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

Joanneum Research is a publicly owned research organization and HEALTH is one of its R&D institutes. We perform applied science and services for pharmaceutical companies. In this area, we act as a boutique Clinical Research Organization (CRO) and our services include preclinical and clinical pharmacokinetic, pharmacodynamic, and bioequivalence studies at the target tissue level.

Furthermore, we conduct in-vitro release testing (IVRT) and customized bioanalyses (PK, PD) in a GLP certified lab. Other services include metabolomics, data management, biostatistics, and medical writing.

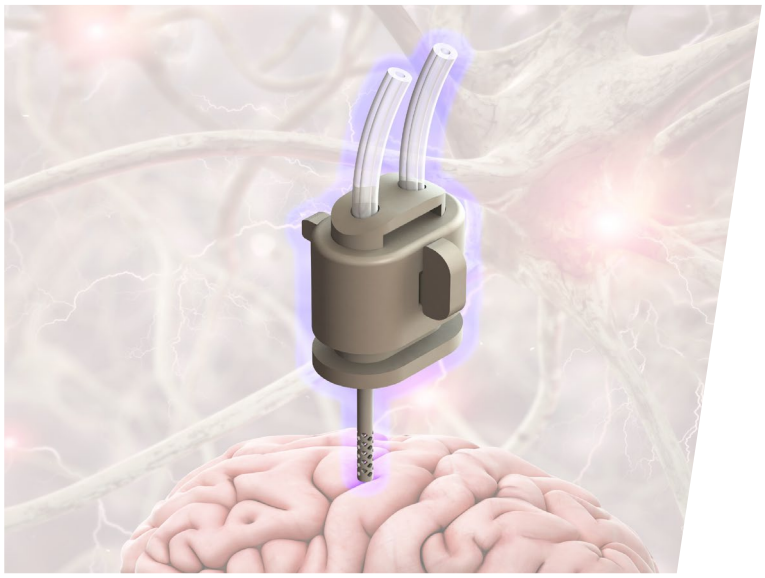
Who We Are

Open Flow Microperfusion (cOFM)

Dermal Tissue **Adipose Tissue** **Brain Tissue**

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

We support clinical and preclinical research and development activities of our customers with our unique and patented Open Flow Microperfusion (OFM) technology. OFM provides unfiltered, merely diluted interstitial fluid for unique insights into metabolism and signaling as well as pharmacokinetics and pharmacodynamics at the target tissue. OFM is applicable in different target tissues. It is well established in dermal, adipose, and brain tissue. Currently we also have projects of first use in muscle and mucosa tissue. We perform preclinical ex-vivo and in-vivo studies as well as clinical studies. Dermal OFM and adipose OFM are CE-certified and are used in clinical studies, mainly for PK/PD/BE studies.



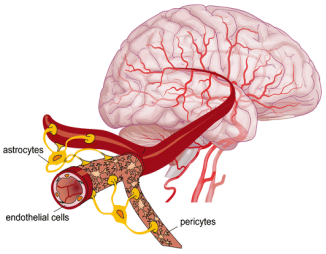
Biomedical Tissue Monitoring in the Brain

This presentation is focused on the cerebral application of OFM – cOFM.

Biomedical Tissue Monitoring

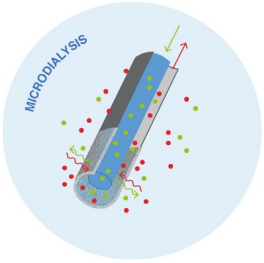
How to Measure Substances in the Brain In-Vivo?

Blood-Brain Barrier (BBB)

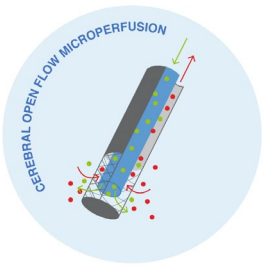


Credit: https://www.researchgate.net/figure/Brain-vasculature-separates-the-circulating-blood-from-the-CNS-tissue-The-vessels-are_fig1_320026611

Microdialysis



cOFM



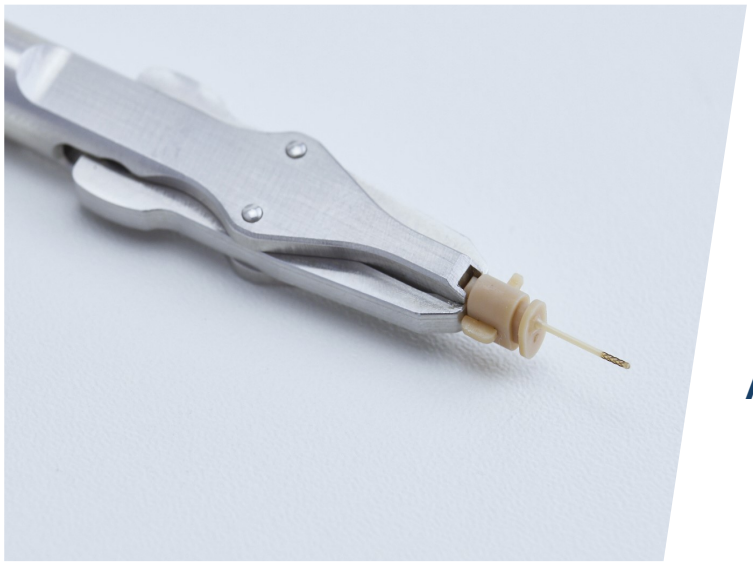
Credit: JOANNEUM RESEARCH

Features:

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

Measuring substance concentrations in the brain is hindered by the blood brain barrier, which is a highly selective barrier protecting the brain from harmful substances. Thus, substance concentrations that are measured in blood samples do not necessarily reflect substance concentration in brain tissue. Implantation of any cerebral probe in the brain causes a rupture of blood vessels and a local destruction of the blood brain barrier that leads to confounded concentration measurements due to blood brain barrier leakage.

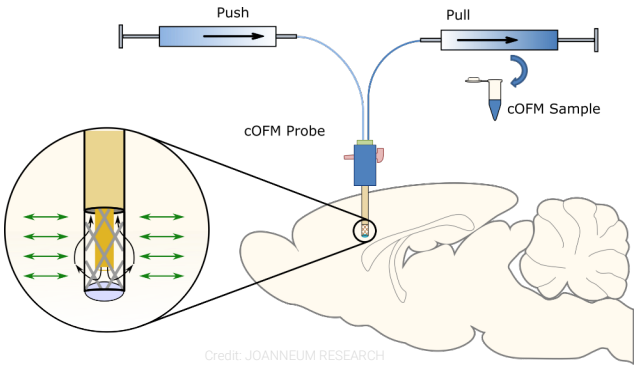
This clearly poses an obstacle to drug development for neurological diseases. Two in-vivo methods exist to measure substances in the brain of live animals and we are using both routinely in our facility: microdialysis and cOFM. The basic concept of both methods is the same: A probe is implanted in the brain and then perfused with a physiological fluid. Substances from the brain interstitium are diffusing into the probe and are transported out of the brain for subsequent bioanalysis. Microdialysis uses a membrane through which the substances have to diffuse before they reach the inner probe lumen.
Material and pore size of the microdialysis membrane can be selected to exclude or include molecules of different sizes, charge and lipohilicity. Considering protein binding, microdialysis can be used to sample the free fraction of a substance that is not protein bound. In contrast cOFM has no membrane and therefore no exclusion regarding size, charge and lipophilicity. In other words, OFM samples unfiltered cerebral interstitial fluid, including also the protein bound fraction of a substance. Additional features of the cOFM are the sampling of cerebral interstitial fluid with an intact blood brain barrier resulting in no contamination from the blood, and cISF can be sampled long-term, meaning continuous sampling over several days. The following slides show more details of cOFM technology and also include a case study comparing microdialysis and cOFM when sampling the small lipophilic drug amitriptyline.



Cerebral Open Flow Microperfusion

Cerebral Open Flow Microperfusion

Concept

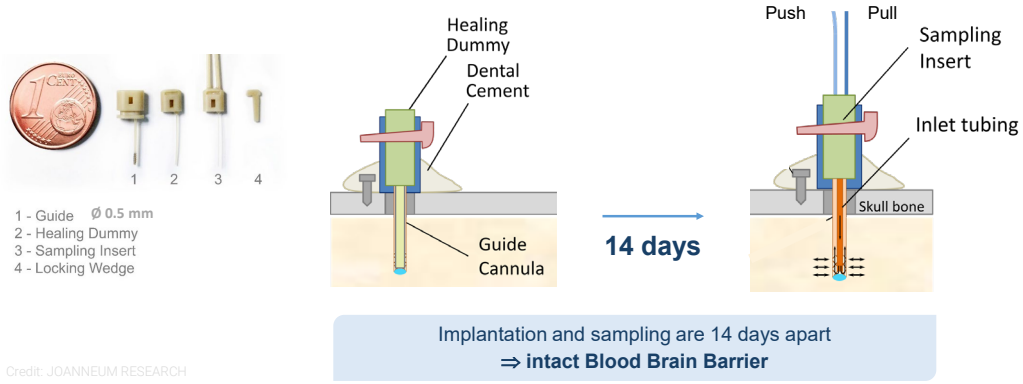


cOFM is a unique tool to investigate pharmacokinetics (PK) and pharmacodynamics (PD) in the brain with an intact BBB.

cOFM is a tool to sample cerebral interstitial fluid for the investigation of PK and PD in the brain with an intact BBB. This slide shows the basic concept of cOFM sampling. The cOFM probe is implanted into the brain region of interest. During sampling, the cOFM probe is continuously perfused with a physiological fluid - the perfusate. The fluidic flow can be achieved by a push and a pull syringe, but we use a precise peristaltic pump to realize a continuous flow through the cOFM probe. The enlargement shows the exchange area at the tip of the probe. The perfusate exchanges molecules with the cerebral interstitial fluid (ISF) along the exchange area, resulting in diluted and also unfiltered cerebral ISF samples, as no membrane is involved. The cerebral ISF samples are collected in vials and contain all molecules present in the brain tissue without restriction due to size or inherent chemical properties and include drugs, proteins and antibodies and even larger structures like nanoparticles.

cOFM Cerebral Open Flow Microperfusion

Implantation and Sampling



Credit: JOANNEUM RESEARCH

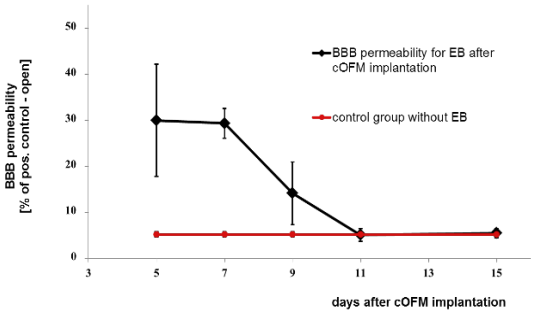
Implantation and sampling are 14 days apart
⇒ **intact Blood Brain Barrier**

On this slide the left image shows the components of the cOFM and the size in relation to a 1 Euro cent coin. The part of the probe which is inside the brain is 0.5 mm in diameter. On the image in the middle you can see the implanted cOFM guide cannula with a healing dummy inside. As the implantation of a probe into the brain causes rupture of blood vessels and local destruction of the BBB we allow the BBB to heal and to re-establish for 2 weeks after probe implantation. For the actual sampling, shown on the right side, the healing dummy is removed and the sampling insert is placed inside the guide. Pushing the sampling insert into the guide does not cause a second trauma to the brain and the blood brain barrier, because the sampling insert stays within the guide cannula. Therefore, cOFM sampling can be performed with an intact blood brain barrier.

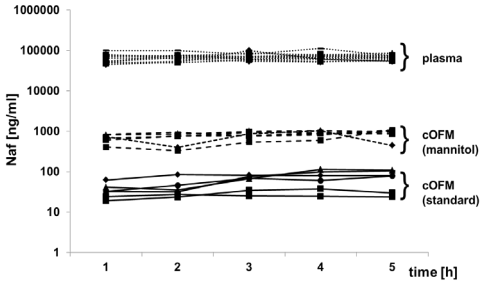
cOFM Cerebral Open Flow Microperfusion

Intact Blood Brain Barrier

Evans Blue BBB permeability



BBB marker – Sodium fluorescein (Naf)



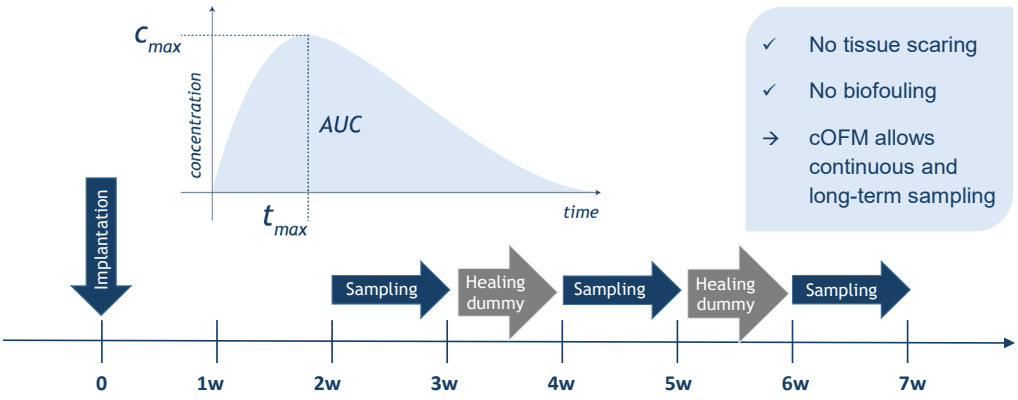
Birngruber T, Ghosh A, Perez-Yarza V, Kroath T, Ratzer M, Pieber TR, et al. Cerebral open flow microperfusion: a new in vivo technique for continuous measurement of substance transport across the intact blood-brain barrier. Clin Exp Pharmacol Physiol 2013;40:864–71. https://doi.org/10.1111/1440-1681.12174.

We have investigated the permeability of the BBB using Evans blue and sodium fluorescein. The graph on the left shows the integrity of the BBB on different days after implantation. Evans blue was injected i.v on different days after probe implantation and fluorescence of the brain tissue was measured. The black line shows the (Evans blue) fluorescence of brains with implanted cOFM, which was compared to the baseline fluorescence of brains with cOFM but without Evans blue injection, here shown by the red line. The permeability for Evans blue was high for 5 days after probe implantation, then starting to decline on day 7 and after day 11 no permeability was observed after probe implantation. Hence, we concluded that the BBB needed 11 days to re-establish after cOFM implantation trauma.

The graph on the right shows the permeability of the BBB 15 days after implantation when challenged with mannitol. Mannitol creates an osmolarity gradient, which causes the endothelial cells to shrink and thereby opens the BBB. We continuously infused the small marker sodium fluorescein (NaF) and reached stable plasma concentration, which you can see in the top lines. cOFM sampling was performed for 5 hours. NaF is able to pass the BBB. cOFM samples with standard perfusate contained low and stable levels of NaF, which is the bundle of lines at the bottom of the graph. In one group we added hyperosmolaric mannitol to the perfusate to open the BBB locally and in this group we observed a higher concentration of NaF in the cOFM samples. This experiment confirmed that i) standard cOFM sampling does not open the BBB and ii) the BBB is closed at 15 days, but can be opened with mannitol.

Cerebral Open Flow Microperfusion

Time-Resolved Substance Concentration Monitoring



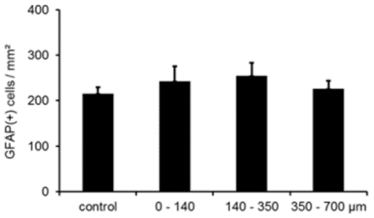
- ✓ No tissue scaring
- ✓ No biofouling
- → cOFM allows continuous and long-term sampling

cOFM sampling provides time resolved concentration profiles of the substance of interest in the brain region of interest, with the possibility to observe a complete PK profile or follow biomarkers in response to therapy in the same animal for days, weeks, and theoretically for months. The absence of a membrane prevents biofouling, which opens the opportunity for prolonged sampling periods. We have already performed continuous sampling for 7 days. The time line on the bottom of this slide shows a theoretical example in which sampling and resting can be alternated. During resting, a new healing dummy has to be inserted until the next sampling.

cOFM Cerebral Open Flow Microperfusion

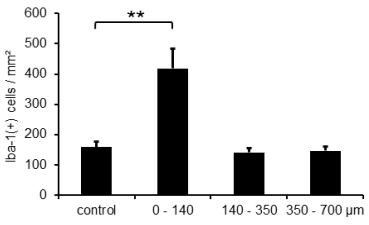
Minimal Tissue Reaction

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

Birngruber T. Long-term implanted cOFM probe causes minimal tissue reaction in the brain. PLoS One 2014;9:e90221–e90221. https://doi.org/10.1371/journal.pone.0090221.

This slide shows the results of our investigation of the tissue reaction to the cOFM probe. The graph on the left shows the quantification of astrocytes at different distances to the probe 15 days after probe implantation at which the BBB is reestablished and sampling can be started. The number of astrocytes was not significantly different compared to the control, hence no glial scar. The right graph shows the quantification of macrophages also at different distances to the probe. Here we found a mild increase in the closest vicinity to the probe. A second evaluation was performed 30 days after probe implantation. As the results were the same, the data is not shown here. In summary, cOFM causes only minimal tissue reaction and the tissue enters a stable condition 15 days after probe implantation.

cOFM Cerebral Open Flow Microperfusion

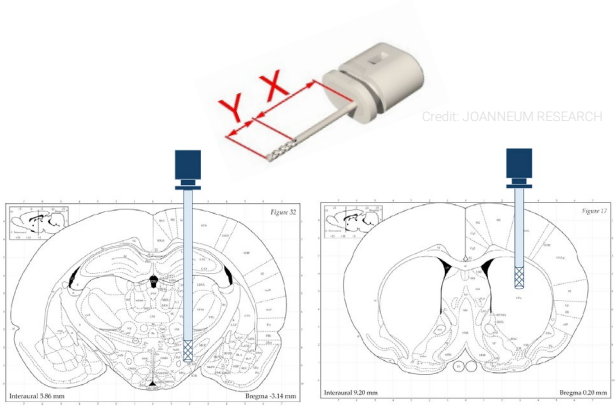
Different Brain Regions

ISF sampling in

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

CSF sampling in

- lateral ventricle
- third ventricle



cOFM can be configured to sample cISF from different brain regions. The length of the probe and the length of the exchange area, as indicated by X and Y in the top figure, can be adapted to reach the target brain region. For example striatum, cortex, hippocampus, hypothalamus and others. Also, cOFM can be used to sample CSF (cerebrospinal fluid) from the lateral ventricle or third ventricle. Depending on the brain region of interest up to three probes can be placed in a rat brain. Typically, we use 2 probes, one in the brain region of interest and on the other hemisphere one in the ventricle, which allows direct comparison of drug concentration in ISF and CSF.

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 ß, tau, albumin, IGGs, …)
  - Nanoparticles (doxorubicin, liposomes, …)
- **Hormones**
  - Leptin (16 kDa), GLP-1 (3297 Da)
- **Biomarkers**
  - Cytokines, eicosanoids, growth factors, glucose, …

This slide shows you examples of what we have already sampled with cOFM. cOFM is able to sample lipophilic substances with logP higher than 4 such as amitryptiline. Also, larger molecules such as antibodies, proteins or even nanoparticles present no problem. We have successfully monitored the hormones leptin and glucagon-like peptide for the development of new drugs fighting obesity. As the cOFM sample contains all substances present in the interstitial fluid, it is possible to measure a portfolio of biomarkers, like cytokines, eicosanoids, growth factors, glucose, etc.

cOFM 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

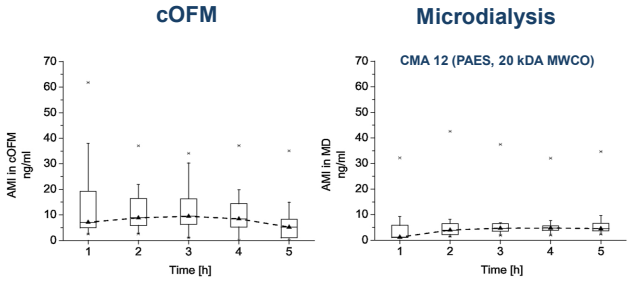
cOFM is perfectly suited to
i) monitor BBB function and permeability changes
ii) monitor substance transport across the intact BBB and provide time-resolved concentration profiles of substances in the brain.
ii) Further, and actually even in parallel, cOFM can monitor the pharmacodynamic response to treatment, measuring biomarkers such as leptin, tau or amyloid ß as well as other proteins in the brain.

In summary, cOFM is a tool for PK/PD studies in the brain supporting drug development, currently at a pre-clinical stage. It is well established and routinely used in rodents, both mice and rats, with the possibility to use transgenic animals. Ongoing development is the use in larger brains, including pigs, non-human primates and finally human. First experiments in non-human primates have already been successfully performed.

Case Study

Amitriptyline Sampling with cOFM and Microdialysis

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



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, Ratzer 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.

This case study compared the cOFM and microdialysis sampling of a small but lipophilic neuroactive drug, the antidepressant amitriptyline. Amitriptyline was injected i.p. and ISF was sampled with cOFM from the one hemisphere and with microdialysis from the other hemisphere. The microdialysis membrane had the same diameter and length as the cOFM exchange area, and both probes were perfused with the same perfusate for optimal comparison. The graph on the left shows the concentration-time profile of amitriptyline in the brain ISF sampled with cOFM. The graph on the right shows the concentration-time profile for microdialysis.

The table shows the AUC of the concentration time curves. The values in the blue box indicate that cOFM results were twice as high as MD results. The red boxes highlight metabolites that were only detected with cOFM but not present in microdialysis samples. This experiment showed that for this small lipophilic substance only cOFM was able to detect low amounts of analytes due to the absence of a membrane and less adsorption.

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 Prieult 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)

cOFM has been used for the development of drugs in neurodegenerative diseases and obesity/diabetes. Further, it was used to investigate the BBB integrity during systemic inflammation and to test the transport of nanoparticles across the BBB.

Here is one publication for further reading for each topic. We are currently developing a cOFM glioblastoma model to measure pharmacokinetics and pharmacodynamics inside the tumor.

Cerebral Open Flow Microperfusion

cOFM service @ JR HEALTH

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

.

At Joanneum Research HEALTH we provide a number of services in combination with cOFM technology. The scheme on the right shows all steps and materials involved in a cOFM experiment. cOFM probe production is done in house which enables us to quickly develop customized configurations. Implantation is performed by specialized animal surgeons and we offer training either in our facility or in your facilities. For continuous sampling we use our in-house developed precise peristaltic OFM pump including appropriate tubing that can quickly be adapted to specific analyte requirements. Our current cOFM setup includes the BASI raturn to sample in awake and freely moving animals, as well as the BASi culex for blood sampling in parallel to cOFM sampling.

In summary, the full setup allows dosing and simultaneous sampling of cISF, CSF and blood in awake and freely moving animals over several days. Having developed cOFM we can provide both, scientific as well as technical advice for cOFM studies. Another major strength is the quick adaptation of the complete system to specific research questions and support with the design of a cOFM study that helps our customers to make informed decisions early in their drug development program.

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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