Application note 2

on the microdialysis technique. Reprint from Microdialysis Academy, Microline no 1

Designing the MICRODIALYSIS EXPERIMENT

Microdialysis gives you a "sneak preview" of what goes on in the body - before any chemical events are reflected in changes of systemic blood levels or even whole tissue levels. The microdialysis probe is designed to mimic a blood vessel and by keeping this metaphor in mind, it is easy to conceive of the many ways you can take advantage of the technique.

The microdialysis probe mimics a "blood vessel"

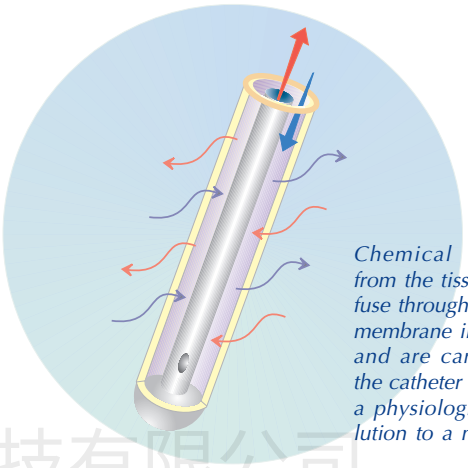
The microdialysis pump is the "heart", pumping a physiological salt solution, "the blood", through the "vessel" i.e. the microdialy-sis probe. The advantage of this heart and vessel is that *you* control it and you can collect as many samples as you like without depleting the animal of any blood. Furthermore, you know exactly where the vessel is located, in which tissue, organ or even brain nucleus. You can perform your sampling in the anaesthetized animal as well as in the freely moving animal and still inflict minimal harm. The fact that samples can be taken repeatedly from the same animal minimizes the number of animals needed in an experiment.

The microdialysis pump is the "heart"

A microdialysis probe is usually a concentric tube where the perfusion fluid enters through an inner tube, flows to its distal end, exits the tube and enters the space between the inner tube and the outer dialysis membrane.

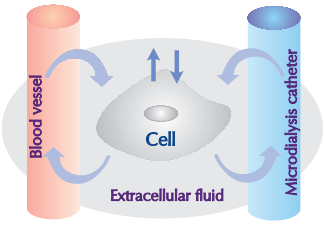
The direction of flow is now reversed and the fluid moves toward the proximal end of the probe. This is where the "dialysis" takes place, that is the diffusion of molecules between the perfusion fluid and the extracellular fluid.

It is important to realize that this is an exchange of molecules in both directions. The difference in concentration over the membrane dictates in which direction the gradient is established. You may collect an endogenous compound at the same time as you introduce an exogenous compound, e.g. a drug, into the tissue.



Chemical substances from the tissue fluid diffuse through the dialysis membrane into the tube and are carried out of the catheter by a flow of a physiological salt solution to a microvial.

The *gradient* of a particular compound depends upon the difference in concentration between the perfusate and the extracellular fluid but also upon the velocity of flow inside the microdialysis probe. The *absolute recovery* (mol/time unit) of a substance from the tissue depends on (1) the "cut off" of the dialysis membrane (usually defined as the molecular weight in Dalton at which 80% of the molecules are prevented from passing the membrane), (2) the length of the membrane, (3) the flow of the perfusion fluid and (4) the diffusion coefficient of the compound through the extracellular fluid. The reverse holds true for substances entering the tissue from the probe.



The following is a simple checklist that may help you design your microdialysis experiment:

1. ***Properties of the probe membrane.*** A membrane with low molecular weight cut off purifies your sample by excluding large molecules, while a high molecular cut off recovers large substances, such as peptides or proteins.

2. ***Length of the membrane.*** A longer membrane gives a better recovery of the substances you are interested in but the choice is usually limited by the size of the structure you want to study.

3. ***Perfusion flow.*** A high flow if you want to remove or introduce as many molecules as possible per time unit and a low flow if you want to obtain a more concentrated dialysate. It is worth considering that a high flow is liable to disturb the physiology simply because more substances are removed.

4. ***Composition of the perfusion fluid.*** Ideally it should be as close as possible to the composition of the extracellular fluid. However, you may want to change the concentration of sodium, potassium or calcium in order to influence the membrane function in the region you are studying.

5. ***Type of probe.*** A stiff probe is suitable for a stereotaxic experiment on the rat brain while a flexible probe may be suited for dialysis in a peripheral organ such as adipose tissue, muscle, liver or kidney. A probe in the brain may require a preimplanted guide cannula while a subcutaneous probe may be implanted an hour or so before the start of the experiment.

6. ***Time needed to obtain steady state conditions.*** The introduction of a probe into the tissue will always cause damage and the recovery of function will take a certain time period. An hour is often used to reach "baseline conditions".

7. ***Does the animal have to be awake*** or can it be kept under anaesthesia? Using awake animals does not neccessarily mean that the conditions are more "normal". An awake animal is subject to pain and stress that may influence the results as much as the anaesthesia.

8. ***Design of a control experiment.*** This is certainly one of the most important parts of any experimental design. You will have difficulties in determining the influence of a great number of known or unknown variables in your experiment, however, a well designed control experiment will take care of many of these problems.

9. ***Dose reponse experiments.*** Microdialysis is a wonderful technique for studying drug actions. The ease by which one can follow the time course of local drug concentration in the tissue and drug effects on local physiology is one of the really strong points of the technique. However, it is surprising how few publications include a dose response study - especially as we know that the qualitative action of a drug often changes as the dose changes.

10. ***Chemical analysis technique.*** Does it require a small sample volume and a high concentration (e.g HPLC) or a large sample volume and a high amount of the particular compound (e.g. RIA - Radio Immuno Assay). This means that you may want to choose a low and a high perfusion flow, respectively.

11. ***Time resolution*** needed in your experiment. Frequent sampling usually means higher perfusion flow in order to get enough sample volume for the analysis.

12. ***Instrument set up.*** Do you e.g. need to change the perfusion fluid during the experiment in order to introduce a drug or change the ionic composition of the fluid? In that case you may need a liquid switch or a pump with syringes that can be individually controlled.

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