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Effect of pumping methods on transmembrane pressure, fluid balance and relative recovery in microdialysis

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Abstract

There is a growing interest in using large pore size probes for microdialysis of macromolecular markers to monitor cell and tissue functions. Fluid balance could be an important issue when using large pore size microdialysis probes, which are affected by the mode of operation. In this study, the effect of pumping systems, push, pull, push-and-pull, and the resulting transmembrane pressure on the fluid balance, as well as, the relative recovery of small molecular nutrients and metabolites and macromolecules (proteins) were examined. The validity of the internal reference *in situ* calibration was examined in detail. It is concluded that a push-and-pull system is the only effective method of eliminating fluid loss or gain. The relative recovery of small solutes is not affected much by the applied pumping methods; however, the relative recovery of macromolecules is significantly influenced by them. The *in situ* calibration technique using Phenol Red can provide reliable results for small molecules including glucose and lactic acid. Using 10 and 70-kDa fluorescent dextrans as the internal standard for large molecules *in situ* calibration of similar size does not work for the pull pump system, but does work well when using a push-and-pull pumping method.

Keywords: Microdialysis; Fluid balance; Protein sampling; Relative recovery; Pumping effect; Transmembrane pressure

1. Introduction

Microdialysis is a technique for sampling and analysing soluble molecules in extracellular fluid. It is performed by perfusing a small semi-permeable hollow fibre membrane probe inserted into the tissue with a physiological fluid (the *perfusate*). Molecules outside the membrane in the extracellular fluids will diffuse through the membrane due to the concentration gradient, if they can pass the pores, and will be carried to the outlet by the continuously flowing perfusion fluid. The solution that exits the probe, the *dialysate*, which contains the marker molecules, can be collected for analysis. In conventional microdialysis using small pore sized membrane probes, large molecules will either be completely rejected by the membrane pores or diffuse so slowly through the membrane that their recovery is negligible.

One of the most important parameters in the microdialysis application is the relative recovery (RR), which links the measured concentration in the dialysate to the true concentration to be measured. The relative recovery (RR) of a given molecule in the dialysate is represented as

$$\mathrm{RR} = \frac{C_\mathrm{d}}{C_\mathrm{e}} \times 100\% \tag{1}$$

where C_e is the concentration of a molecule of interest in extracellular fluid, and C_d is that of same molecule in the dialysate. It is important to note that for Eq. (1) to be meaningful the microdialysis probe must remove only small quantities of the molecule of interest form the extracellular fluid such that its concentration remains essentially constant. Physiologically, this is important too as it is undesirable for the sampling to interface the physiological environment around the microdialysis probe.

The mass transfer occurred in the microdialysis probe can be analysed:

$$Q\,\mathrm{d}C = K(C_\mathrm{e} - C)\,\mathrm{d}A \tag{2}$$

where Q is the volumetric flow rate of the perfusate which is assumed to remain constant. K is the overall mass transfer coefficient [1], C the concentration of the molecule of interest in the perfusate/dialysate, and C_e is the concentration of the same

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molecule outside the probe and assumed to be constant.

Integrating Eq. (2) over the length of the probe,

$$\ln\left(\frac{C_e - C_{out}}{C_e - C_{in}}\right) = -\frac{KA}{Q} \tag{3}$$

$C_{in} = 0$ in most cases, and $C_{out} = C_d$ the concentration in the dialysate. Eq. (3) then becomes

$$\frac{C_d}{C_e} = 1 - e^{-(KA/Q)} \tag{4}$$

As indicated in Eq. (4), RR is dependent on the membrane area, A, and perfusion flow rate, Q, and the overall mass transfer coefficient K. However, the overall mass transfer coefficient, K, is difficult to determine, as it depends on the external mass transfer from the extracellular fluid to the membrane surface, the diffusion through the membrane, and internal mass transfer by convection. In particular, the external mass transfer is difficult to evaluate due to variation and uncertainties inside the local tissue structure.

In practice, RR is determined by various calibration methods. One convenient method is the *in situ* calibration using the internal reference technique [2,3]. As described by Scheller and Kolb [3], the relative loss (RL) is a measurement of a marker molecule diffusing from the probe perfusate into the surrounding interstitial fluid. It is

$$RL = \frac{C_p - C_d}{C_p} \times 100\% \tag{5}$$

where C_p is the concentration of a marker in the perfusate and C_d is the concentration of the same molecule in collected dialysate.

Assuming diffusion through the membrane to be bi-directional, i.e. the same in both directions, RL is assumed to be equal to RR if the solute taken as the internal standard has the same or similar molecular size and diffusion properties as the solute of interest. Knowing RR, based on Eq. (1), C_e can be readily determined from the measured C_d.

In the early applications of *in vivo* microdialysis, most studies focused on measurement of small molecules, such as neurotransmitters [4,5], metabolites [6,7] and small molecular drugs [8,9] with microdialysis typically using membranes of a molecular weight cutoff (MWCO) 6–35 kDa to exclude bigger molecules to simplify the analytical procedures. Recently successful attempts have been made to sample protein markers in muscle [10,11], adipose tissue [12], dermis [13] and *in situ* tissue engineering monitoring [14] using large pore size membrane probes.

By collecting associated macromolecules at the action site, microdialysis currently represents the best available technique for monitoring cell functions and their changes [14,15], with simultaneous monitoring cell metabolic activities. However, sampling and analysing macromolecules is highly challenging using microdialysis due to the following

(a) low intrinsic recovery due to the low effective diffusivity of macromolecules;
(b) low concentration of macromolecules themselves in the extracellular fluids;
(c) difficulty in analysing low concentration macromolecules in solution;
(d) adsorption of proteins onto the probe membrane leading to probe fouling;
(e) possibly significant fluid loss when large pore size membrane probes are used.

In practice, it is desirable to minimise the fluid loss or gain and increase the relative recovery of macromolecules such as proteins to make the next stage quantification and assay easier. To minimise the fluid loss through the probe due to the osmotic pressure effect, the osmotic pressure of the perfusate is often adjusted to balance the physiological osmotic pressure by adding an osmotic agents, e.g. dextran-70 [16,17] or a protein such as bovine serum albumin (BSA) [18]. Employing ultrafiltration in the microdialysis procedure, e.g. to create a transmembrane pressure (TMP) and hence a net fluid flow through the membrane, either using a pull pumping system [13,19] or creating a hydrostatic pressure difference [20] can increase the protein recovery. However, this will cause a non-zero permeate flux and the dialysate will gain fluid, which is not desirable for most *in vivo* applications. On the contrary, if a push pump is used, there may be a net fluid loss. Decreasing the flow rate, Q, may achieve a higher recovery (see Eq. (4)) [21]. However, low perfusion flow rates may be hampered by problems associated with sample evaporation as well as poor temporal resolution [22]. Eq. (4) also shows that increasing membrane area will increase the recovery. However, large probes (either length or diameter) will cause increased invasion which is a concern for particularly for *in vivo* application of microdialysis, and also reduce spatial resolution.

2. Microdialysis with different pumping methods

The perfusion into the microdialysis probe can be driven with three different mechanisms: push, pull, push-and-pull, as shown in Fig. 1. Normally a pump is employed to feed perfusate continuously to the probe. This is termed the push pumping system (Fig. 1a). When a push pump is used, the pressure of the fluid within the membrane probe will be higher than that of atmosphere (the dialysate collector). As most experiments are carried out at atmospheric pressure, there exists a net pressure difference across the membrane, i.e. a positive transmembrane pressure (TMP).

The perfusion flow rate is universally low in microdialysis (in the order of μl/min) and the connection tubing diameter is small (less than 1 mm), hence it is safe to assume laminar flow inside the connection tubing. The hydrodynamic pressure at the exit of the membrane probe can then be estimated using Hagen–Poiseuille law, i.e.

$$\Delta P* = 128\,\mu\left(\frac{L}{d^4\pi}\right)Q \tag{6}$$

where μ is the viscosity of the perfusion fluid, and d and L is the diameter and length of the tubing. The pressure profile along the probe can be seen in Fig. 2a.