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Review

Microdialysis of Large Molecules

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ABSTRACT

Microdialysis is a validated and well-established technique for recovering and measurement of exogenous as well as endogenous small molecules in the interstitial spaces of various tissues. Microdialysis of large molecular weight compounds has become possible due to the availability of large molecular weight cutoff membranes and is being explored extensively. There are increasing reports of successful recovery of large molecules such as proteins, cytokines, and neuropeptides using microdialysis. This is not only useful for studying protein expression but also for clinical evaluation of disease biomarkers in different tissues. Large pore microdialysis along with open flow microperfusion offers great promise in determining interstitial tissue concentrations of therapeutic proteins including monoclonal antibodies and helps in understanding their pharmacokinetic-pharmacodynamic relationship.

Introduction

Microdialysis is a powerful technique that enables continuous *in vivo* sampling of substances or analytes from the extracellular tissue space. A probe consisting of a semipermeable membrane is inserted in the tissue and perfusion fluid containing physiological buffer is passed through it at a constant flow rate. The analytes in the interstitial fluid of the tissue can undergo diffusion and ultrafiltration through the membrane and is subsequently collected from the outflow tubing of the probe as shown in Figure 1.[2] Microdialysis is a relatively less invasive technique that is used to measure free, unbound analyte concentrations in the extracellular fluid of virtually any tissue. One of the main advantages of microdialysis is its ability to detect or measure temporal variations in the concentration of analytes in the extracellular tissue space.[3]

In the postgenomic era, an increasing attention has been focused on the structure, function, concentration, and distribution of proteins. These include extracellular cytokines and neuropeptides having a molecular weight of 8-10 kDa and growth factors with a molecular weight of around 30 kDa which are involved in various important physiological and metabolic processes. In order to understand more clearly the mechanisms underlying the initiation and development of human diseases at a local tissue level, it is necessary to be able to gain an insight into both the temporal and the spatial generation or appearance of these proteins.

Although microdialysis is a widely used and well-established technique for determination of free, unbound concentrations of small molecules in tissues, with the availability of large pore membranes it is now increasingly employed to monitor interstitial concentrations of regulatory cytokines and proteins.[4] The objective of this article is to provide a comprehensive overview of the large molecule microdialysis that has been reported since a short review by Clough et al. in 2005.[5]

Principle of Microdialysis of Large Molecules

The principle of microdialysis is based on the Fick's first law of passive diffusion wherein the concentration gradient drives the transfer of solutes across a semipermeable membrane. There is continuous flow of perfusion fluid through the inlet tubing and analyte molecules that are smaller in size than the pores of membrane can enter into the lumen of membrane and come out through the outlet tubing as dialysate. Because analytes larger than the probe molecular weight cutoff (MWCO) are unable to diffuse across the membrane, relatively clean samples can be recovered. Interestingly, MWCO does not present the actual pore size of the membrane but is a statistical measurement of the microdialysis sampling efficiency for molecules of certain size range. For

Abbreviations used: IL, interleukin; NGF, nerve growth factor; VEGF, vascular endothelial growth factor.

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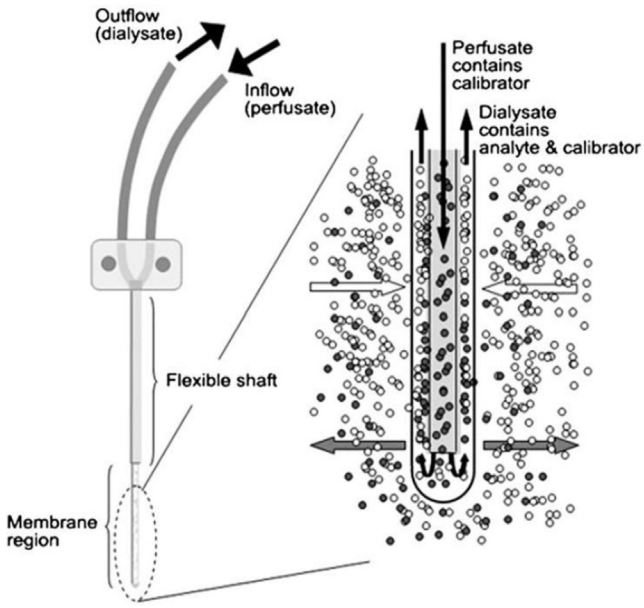

Figure 1. Concentric probe design. The magnified membrane region of concentric microdialysis probe illustrates net diffusion of an analyte of interest (open circles) into the probe, and the net diffusion of the calibrator (closed circles) from the probe to the extracellular space. Reprinted with permission from Chaurasia et al.[1]

example, 20 kDa MWCO membranes will reject 80%-90% of the molecules with a molecular weight of 20 kDa.[6]

With low MWCO membranes (20 kDa or below), the passage of analytes occurs via diffusion mechanism driven by concentration gradient. On the contrary, in high MWCO (100 kDa or above) microdialysis membranes it occurs via ultrafiltration mechanism wherein the analyte molecules are mainly carried through the large pores of membrane by convection.[7] Because of pressure-driven convective flow mechanism, microdialysis membranes with high MWCO are more sensitive to pressure changes.

The performance of the microdialysis sampling is often characterized by fluid recovery (FR) and extraction efficiency (EE). FR is the ratio of the volume of the dialysate collected ($V_{dialysate}$) to the volume of the perfusate that is delivered ($V_{perfusate}$) into the microdialysis probe and expressed as follows: $FR = V_{dialysate}/V_{perfusate}$. Generally, the equilibrium between perfusate and tissue interstitial fluid reservoir is not achieved even at low perfusate flow rate as the perfusate is constantly passed through the probe. So the concentration in dialysate is not the absolute concentration but represents only a fraction of analyte concentration in the tissue or reservoir. This fraction termed as EE is used to calculate the analyte concentration in sampled extracellular space or reservoir using dialysate concentration. The EE is defined as the ratio between the loss and gain of analyte during its movement through the microdialysis probe ($C_{perfusate} - C_{dialysate}$) and the difference between perfusate and reservoir concentrations ($C_{perfusate} - C_{reservoir}$). The EE can be determined by a recovery (EE_R) and delivery (EE_D) experiment. In recovery experiment, EE is reduced to $EE_R = C_{dialysate}/C_{reservoir}$ as the perfusate analyte concentration is zero. In delivery experiment, a known concentration of analyte is added to perfusate while its concentration in reservoir is zero. The EE_D is calculated as $EE_D = (C_{perfusate} - C_{dialysate})/C_{perfusate}$. Ideally, the EE determined by both delivery and recovery experiment should be similar. During *in vivo* microdialysis experiments, the calibration of probe is performed by determining the EE by delivery method and determined recovery is assumed to be same for calculation of concentrations. Alternatively, probe calibration can also be performed by retrodialysis and no net flux (NNF) method. In retrodialysis or reverse dialysis, chemically similar or radiolabeled form of analyte is added as an internal standard to the perfusate and its disappearance from the probe is measured throughout the microdialysis experiment. In the NNF method, different concentrations of analyte, both below and above its concentration in reservoir or sampling site, are consecutively added to the perfusate. When the analyte concentration in perfusate is lower than that of the reservoir, there will be a gain of analyte in the perfusate. When the analyte concentration in perfusate is higher than that of reservoir, there will be a loss of analyte in the reservoir. When the analyte concentration in both perfusate and reservoir is equal, there is NNF of analyte across the membrane. The difference between analyte concentration in dialysate and perfusate samples are plotted versus the original analyte concentration in perfusate samples and the EE is determined from the slope of the resulting line.[1,2]

Challenges and Experimental Factors Affecting Macromolecular Recovery

In order to recover analytes of higher molecular mass from interstitial space, microdialysis probes with high MWCOs are required. For high molecular weight substances such as cytokines and proteins, membrane probes having MWCO up to 3000 kDa have been used. The most commonly used membrane probes have MWCO of 100, 1000, and 3000 kDa. The permeable membranes of an implanted microdialysis probe are mainly made of nanoporous materials constructed from polycarbonate, regenerated cellulose, polyethersulfone, or polysulfone. In addition, the membranes are usually biocompatible and elastic which minimize the host immune response and exert flexible and mechanical property. Most of the materials that are used in microdialysis probes are nonbiodegradable. Microdialysis membranes made of biodegradable or reabsorbable materials may have the advantages of long-term *in vivo* sampling and no additional surgical removal is needed.[8]

Fluid Loss and Recovery

Generally, in most of the microdialysis membranes with low MWCO ($\leq$20 kDa), the number of pores allowing the entry of large molecules such as proteins is small resulting in low EE or relative recovery of large molecules due to an increased mass transport resistance. High MWCO microdialysis membranes typically have large pore sizes which facilitate convection rather than diffusion process and follow ultrafiltration model with regards to the passage of analytes. The ultrafiltration and convective flow mechanism may lead to a leakage of perfusion fluid in the surrounding tissue (reservoir) diluting the interstitial sample and altered FR, thus changing the original concentration of the analytes. This is not favorable and does not represent the actual dynamic process happening *in vivo*.[6]

The large pore size of the high MWCO microdialysis membrane makes it more sensitive to changes in static, dynamic, and osmotic pressures and affects the hydrodynamic equilibrium in the probe.[7] This sensitivity to pressure changes leads to more uncertainties in volume of dialysate and concentration of analyte. Therefore it is important to set and maintain the microdialysis system to obtain an FR as close to 100% and thus avoid leakage of perfusate into the surrounding sampling tissue environment.[9] Typically, the pressure across microdialysis membrane is regulated by the perfusion and osmotic pressure. The perfusion pressure is generally adjusted by changing the perfusion flow rate and by adjusting the height of dialysate collection with respect to the probe membrane position.[10]