Themed Issue: The Role of Microdialysis in Pharmacokinetics and Pharmacodynamics
Guest Editors - Markus Mueller and Ronald J. Sawchuk

Microdialysis of Large Molecules

Submitted: March 25, 2005; Accepted: May 15, 2005; Published: October 26, 2005

Geraldine F. Clough[1]

[1]Infection, Inflammation and Repair Research Division, School of Medicine, University of Southampton, UK

ABSTRACT

Microdialysis has been used in many tissues, including skin, brain, adipose tissue, muscle, kidney, and gastrointestinal tract, to recover low–molecular mass endogenous mediators, metabolites, and xenobiotics from the interstitial space. Recently, molecules of larger molecular mass, such as plasma proteins, cytokines, growth factors, and neuropeptides, have also been recovered successfully using larger-pore membranes. Microdialysis recovery of large molecules offers the opportunity to identify patterns of protein expression in a variety of tissue spaces and to evaluate clinically useful biomarkers of disease. From this may develop a better understanding of the disease process and its diagnosis and more targeted approaches to therapy.

KEYWORDS: microdialysis, proteins, cytokines

INTRODUCTION

Microdialysis is a well-established technique for the continuous sampling of small water-soluble molecules within the extracellular fluid space in vivo. It has an advantage over other sampling techniques in that it can be used to follow temporal variations in the generation and release of a substance at a discreet location within the tissue space. Since its initial use in the brain of experimental animals for the recovery of neurotransmitters and other small hydrophilic solutes,[1] microdialysis has been adapted for use in many other tissues, including skin,[2-9] adipose tissue,[10] muscle,[11,12] and gastrointestinal tract.[13] However, the ability to recover molecules of higher molecular mass has, until recently, been limited by the availability of dialysis probes with high molecular weight cutoffs (MWCOs).

The development of membranes suitable for the recovery of large molecules has been driven by the need to sample the extracellular fluid space for bioactive proteins and regulatory peptides as markers of tissue homeostasis and, more important in a clinical setting, tissue dysfunction and repair. This brief review will consider how recent studies have advanced the understanding of the process by which large molecules are recovered by dialysis and how this may be applied to the use of such probes in clinical settings in the future.

Corresponding Author: Geraldine F. Clough, Infection, Inflammation and Repair, School of Medicine, Mail Point 825, Southampton General Hospital, Southampton SO16 6YD, UK. Tel: +44 (0)2380 794292; Fax: +44 (0)2380 704183; E-mail: G.F.Clough@soton.ac.uk

PRINCIPLES OF DIALYSIS OF LARGE MOLECULES

Some of the first researchers to report the successful recovery of large molecules by microdialysis used a polycarbonate membrane extracted from a plasmapheresis capsule (Plasmaflo, OP-02 Asahi Medical Co Ltd, Kimal plc Bromsgrove, Worcs UK) configured as a linear, flowthrough dialysis probe. These membranes had an MWCO of 300 000 Da and a maximum pore size of 0.3 μm, as stated by the manufacturer. They were used experimentally in both animal models and humans configured as either a linear flowthrough membrane or a concentric dialysis probe, to recover serum proteins, neuropeptides, cytokines, and growth factors.[14-16] Since then, commercially available polyethersulfone microdialysis probes with an MWCO of 6000 to 100 000 Da have been used to recover a wide range of molecular species ranging in size from 3000 to 120 000 Da.[17-20] Some of these studies are summarized in Table 1.

Theoretically, the probes at the higher end of the MWCO range may appear to be satisfactory for the dialysis of cytokines, growth factors, and even high–molecular mass serum proteins. Practically, however, the number of pores within most of the membranes currently used that are capable of allowing the passage of such large molecules is small and results in an increased mass transport resistance and a low relative recovery. For example, the dialysis efficiency (E_d) of a 10 000-Da protein across a commercial 100 000-Da MWCO membrane perfused at a flow rate of 1 μL/min is typically below 5%.[30]

There are also several experimental factors, including probe perfusion rate and duration, that may significantly affect macromolecular recovery. This is illustrated by the in vitro dialysis of serum albumin (67 kDa) using a plasmapheresis membrane configured as a linear probe of 1.5 cm length, where E_d decreases exponentially from 1.01 ± 0.02 at a perfusion rate of 0.1 μL/min to 0.17 ± 0.06 at perfusion rate of >5 μL/min (Figure 1). The dialysis efficiency for a molecule the size of serum albumin may be similarly compromised

Table 1. Examples of Microdialysis Recovery of Large Molecules*

Molecule	Tissue	Reference
IL-1, IL-6	Brain	Woodroofe et al[21]
IL-1β, IL-6, NGF	Brain	Winter et al[15,16]
IL-6	Skin	Sjogren et al[22]
Total protein, neuropeptides	Skin	Schmelz et al[14]
Albumin	Skin	Fellows et al[23]
IL-6, MCP1	Peritoneum	Riese et al[24]
VEGF	Breast	Dabrosin[17,25]
TNFα	Bone	Brown et al[26]
IL-1α, IL-1β, IL-6, M-CSF, VEGF	Reproductive tract	Licht et al[27]
IL-1β, IL-6, TNF, MCP1	In vitro	Trickler and Miller,[28] Ao et al[29]
Dextrans 3000 to 150 000 Da	In vitro	Schutte et al[30]

*IL indicates interleukin; MCP1, monocyte chemotactic protein-1; M-CSF, macrophage colony-stimulating factor; NGF, nerve growth factor; TNF, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

in vivo. This is evidenced by the fall in steady-state protein concentration recovered in dialysate from healthy skin from a value of 0.73 ± 0.30 mg/mL at a perfusion rate of 5 μL/min to 0.47 ± 0.19 mg/mL at 10 μL/min ($P < .005$) (Fellows et al, unpublished data, 2005). However, while the dialysate protein concentration was lower at the higher perfusion rates, the total amount of analyte recovered over a 30-minute perfusion period was greater at the higher perfusion rates (192 ± 14 μL and 225 ± 38 μL at 5 and 10 μL/min, respectively), suggesting that it may be an interesting compromise between the demands of an assay for total amount of material and its requirements for a given minimum concentration or volume.

It should also be noted that continuous perfusion of large-pore dialysis membranes for long periods results in an apparent fall in analyte recovery, with initial total protein levels significantly higher than those recovered in later samples (Figure 2).[23] One explanation for this is that the amount of material available for dialysis recovery falls with time. It is well recognized that the concentration gradient of any solute within the tissue space within the proximity of the probe will be determined by the rate at which it is supplied by, and diffuses through, the tissue and also by the rate at which it is removed in the dialysate. This phenomenon may be particularly true for larger, more slowly diffusing molecules at low biological concentrations within the tissue space.

Taken together, these factors suggest that for high–molecular mass solutes it is unlikely that even at very low rates of perfusion a diffusion equilibrium will be reached and a sample representative of the biological concentration of the analyte will be recovered. Figure 2 suggests that the concentration of total protein outside the probe calculated from the relationship

$$C_{\text{tissue}} = C_{\text{outlet}} / (1 - e^{-PS/F}) \quad (1)$$

where C_{tissue} and C_{outlet} are the concentration of the solute in the tissue and outflow of the probe, PS is the permeability–surface area product for the dialysis membrane, and F is the perfusion rate[31] is initially between 25 and 30 mg/mL but falls to 4 to 6 mg/mL after extended periods of perfusion. Although the initial values of total protein in dialysate approximate well to the published values for interstitial

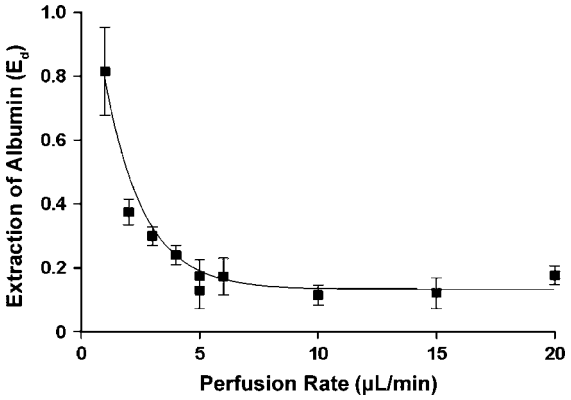

Figure 1. In vitro dialysis efficiency (E_d) of bovine serum albumin (67 kDa) using a plasmapheresis membrane configured as a linear probe of 1.5 cm length perfused with physiological Ringer's solution (pH 7.3). Data are mean ± standard error of mean. from 3 to 5 probes at each flow rate. E_d is calculated as concentration in probe outflow/concentration in external medium.

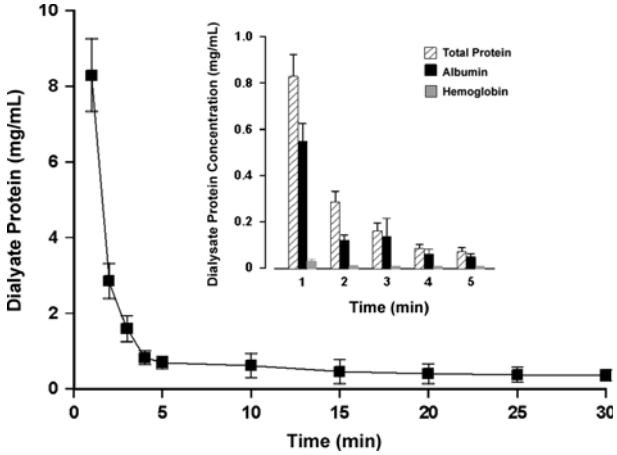

Figure 2. Effect of continuous perfusion on recovery of total protein from healthy human skin using a 300 000-Da large-pore dialysis membrane. The probe was perfused at 3 μL/min with physiological Ringer's solution, and samples were collected initially at 1- minute intervals and then at 5-minute intervals. Data are mean ± SEM from 8 volunteers. Insert shows the relative proportions of albumin and hemoglobin in the dialysate samples collected over the first 5 minutes of perfusion.

protein content of between 12 and 30 mg/mL sampled using micropuncture, interstitial wicks, or blister fluid,[32-34] the absolute amount of any analyte present in the tissue space cannot truly be estimated without calibration of the probe.

Another factor that will influence recovery of solute from the interstitial space is tissue hydration. Recovery may be attenuated by the loss of fluid by ultrafiltration across the highly porous membrane, particularly at high flow rates. Fluid loss into the tissue, as well as having the potential to compromise tissue function and stimulate changes in the tissue levels of the target molecules, may also dilute the content of the interstitial space. An increase in tissue water will reduce further the often low concentrations of the target molecules within the tissue space and may lead to an underestimate of the concentration of the molecules of interest. This is seen when the performance of large pore membranes placed at different tissue sites is compared. We have found that using the linear 300 000-Da MWCO membranes in healthy human skin, both the volume recovery and the total protein concentration of dialysate from probes placed on the ventral surface of the forearm, close to the wrist, differ significantly from those measured using probes located more proximally. In these experiments on healthy volunteers, relative volume recovery measured over ten 3-minute collections fell from 1.04 ± 0.04 in probes placed in the upper forearm to 0.92 ± 0.05 in those placed distally close to the wrist ($P < .02$) (Clough et al, unpublished observations, 2005).

Several groups have attempted to overcome fluid loss into the tissue and to improve total volume recovery by the addition of osmotic agents to the probe perfusate.[28,35] These agents have included serum albumin and modified starches, both of which tend to reduce fluid loss into the tissue space. Some of the additives used have the added advantage of reducing nonspecific adsorption of analyte onto the material of the probe or act as binding agents to stabilize and enhance recovery of hydrophobic or highly tissue-bound molecules.

The ability to recover hydrophobic molecules by dialysis appears compromised by their adsorption onto the polymeric materials used to construct the probe and onto the membrane itself.[36,37] Consequently, the in vitro recovery of several cytokines, including interleukin 2 (IL-2), IL-4, IL-5, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNFα), has been reported to be as low as 1%.[25,29] For other cytokines, in vitro recovery efficiencies of dialysis using a 300 000-Da membrane have been reported to be somewhat higher: 45% ± 8%, 28% ± 4%, and 22% ± 8% for IL-6, IL-1β, and nerve growth factor (NGF), respectively, compared with 17.5% ± 6% for serum albumin.[38] Recovery of cytokines has been enhanced experimentally by the addition to the probe perfusate of albumin, modified starches (cyclodextrins), or lipids.[9,19,22,28,39,40] While the ability of these agents to increase recovery varies considerably and in vitro appears to depend on the nature of the dialysate membrane and the analyte,[9] E_d may be enhanced such that in vivo, a cytokine that is undetectable using a saline perfusate is recovered in measurable amounts (10^{-12}-10^{-9} M) when bovine serum albumin (BSA) is added to the perfusate at concentrations of between 3.5% and 10% (mass-to-volume ratio).[28] Addition of antibody-coated microbeads to the probe perfusate can similarly enhance cytokine recovery by up to 1000% in vitro by trapping cytokine molecules and increasing the diffusive driving force across the probe membrane.[29] This has been particularly effective for TNFα and IL-5, 2 inflammatory cytokines that have proved particularly difficult to recover using saline perfusion of 300 000-Da membranes in vivo (Clough et al, unpublished data, 2005). Whether these recovery enhancers are as effective in vivo as they appear to be in vitro has yet to be explored.

In summary, the consequence of these various physical restraints alone or in combination is that the amount of high–molecular mass material recovered in dialysate, even under optimal conditions, is frequently very low. Furthermore, the concentrations recovered in dialysate may be unrepresentative of tissue levels. Evidence for changes in macromolecular analyte levels from baseline may thus be more effectively sought than absolute values.

Analysis Platforms for Large Molecules in Dialysis Samples

The future of the application of microdialysis to the monitoring of large molecules as markers of disease in a prognostic or diagnostic setting depends very much on the ability to detect and assay them with sufficient sensitivity and reproducibly. While the analytical methods used to detect low–molecular mass solutes in dialysate have been extensively reviewed,[41,42] there are fewer reviews of the analysis platforms suitable for the detection of proteins in dialysate.[43]

As described above, the concentration of many of the proteins of interest is very low, in the nanogram to picogram per milliliter range. Until recently, the majority of studies have employed sensitive fluorometric or radio or enzyme immunoassays, many used at the limits of their detection. The volume requirements of these assays are considerable and limit the number of proteins assayable within a single dialysate sample.[16,22] Sensitive temporal resolution of changes in protein levels at a given site within the tissue space may also be precluded if low perfusion rates and extended collection times are required. The advent of multiplexed cytokine arrays where 10 or more cytokines can be assayed using a 25 to 50 μL sample volume with a similar sensitivity to that of conventional immunoassays, has to some extent overcome this.[43] In Lee et al (unpublished data, 2004) own hands they have proved a useful platform for the analysis of the differential cytokine production in dialysate

and in human skin with a spatial resolution of millimeters and temporal resolution of minutes.

Mass screening tools such as the recent proteomic approaches using mass spectrometry have been used to evaluate patterns of protein expression in a variety of tissue fluids and to explore clinically useful biomarkers of disease.[45] These fluids include plasma, cerebrospinal fluid,[46] and urine.[47] Plasma is the body fluid most extensively assayed because of its accessibility in which more than 175 candidate biomarker proteins with associations with cardiovascular disease have already been characterized.[45,48,49] Cerebrospinal fluid has been probed using 2D gel electrophoresis to detect changes in protein expression patterns in patients with neurological or psychological disease.[46] 2D gel electrophoresis in combination with mass spectrometry has also been used to investigate protein patterns in human cerebral microdialysate (see below).[18] In addition, cerebral dialysate in rats has been screened for endogenous neuropeptide generation using capillary liquid chromatography–tandem mass spectrometry.[20] These approaches, by which many proteins and peptides can be screened at one time, lend themselves to the identification of novel proteins whose release can be correlated with different physiological, pathophysiological, or behavioral states. It is probable that the advent of commercial probes suitable for the recovery of large molecules will result in an increase in the application of such proteomic approaches to microdialysate samples. The analytical challenges, just as in plasma, will be (1) the depth of the tissue fluid proteome, in which concentrations of protein biomarkers vary over many orders of magnitude; and (2) the breadth of the tissue fluid proteome, with potentially many hundreds of bioactive markers.

Microdialysis Sampling of Proteins in Skin

Various techniques have been used to sample the interstitial space in skin and to explore the changes in interstitial protein content under both physiological and pathophysiological conditions. These include the wick technique and the blister suction technique, which have been widely used in the skin for dermatological and pharmacokinetic studies.[33,50,51] They yield values for total interstitial protein concentration of between 18 and 27 g/L, or ~30% to 40% of that measured in plasma. Of this, 13 to 17 g/L is albumin.[32] Although recent studies have shown that there is a close correlation between the 2 techniques when used to sample the subcutaneous interstitial space in healthy human volunteers, neither particularly lends itself to monitoring rapid changes in tissue protein levels. Several groups have now used microdialysis to follow both spatial and temporal changes in interstitial fluid protein content in resting and provoked skin, as discussed earlier.[6,52] Steady-state basal interstitial protein concentration (calculated assuming an E_d of ~20%) appears somewhat lower than that reported using other techniques. However, the protein content of the samples collected within the first 2 minutes of the start of perfusion are more than 5 times higher than this and close to those recovered in blister fluid.[33] Similar or greater levels are also recovered during dermal provocation with agents known to induce plasma extravasation.[53,54]

Microdialysis has been used to follow the upregulation of inflammatory cytokines within the interstitial space in normal and inflamed skin.[22,24,55] It has also been used in breast tissue to investigate changes in angiogenic factors such as soluble vascular endothelial growth factor (VEGF) in health and during tumor angiogenesis[17,56] and to monitor changes within the environment of both acute and chronic wounds.[57,58] The wound environment has proved quite difficult to sample because of the nature of the wound exudate. In exudating chronic ulcers, the average dialysate protein concentration was found to be 0.5 mg/mL (Avery et al, unpublished data, 2005). By comparison, the concentration of protein in chronic wound fluid obtained by direct sampling ranges from 26 to 51 mg/mL, with a mean (± standard deviation) of 38 ± 13 mg/mL, suggesting that dialysis efficiency for total protein is very low under these conditions. Interestingly, volume recovery was also much reduced, with less than 60% recovery using a 2-cm dialysis length of 300 000-Da plasmapheresis membrane configured as a loop to lie within the wound environment.

Recovery and detection of some markers of inflammation anticipated to be present in inflamed skin, including TNFα and IL-5, have proved problematic. Whether this is due to a reduced availability or concentration within the environment of the probe or due to adhesion of the bioactive molecule onto the material of the probe has yet to be confirmed either in or ex vivo.

Microdialysis of Large Molecules in the Brain

Intracerebral microdialysis has been used extensively as a research tool in the investigation of the neurochemical and metabolic changes that occur following acute brain injury. However, few studies have pursued the recovery of larger molecules, particularly cytokines, from the brain by microdialysis. Studies that have attempted to investigate cytokine levels in the traumatized brain have generally been limited to measurements within the cerebrospinal fluid and serum of patients. Recently, we have reported that it is possible to recover high–molecular weight molecules from the parenchyma of the frontal lobe of the brain using a concentric version of the 300 000-Da MWCO microdialysis probe.[16,38] Samples were collected at intervals for periods of several hours during the patients' stay in the intensive care unit. We found that the levels of IL-6 measured in outflowing

dialysis fluid from 13 patients with traumatic brain injury correlated with survival outcome and were between 60 and 1230 pg/mL. NGF levels were between 180 and 2964 pg/mL, with a tendency for NGF levels to be higher in nonsurvivors. IL-1β (0–140 pg/mL), by contrast, showed no association with clinical measures. Interestingly, total protein and albumin levels measured in dialysate remained relatively constant over periods of up to 5 days. Thus, it seems unlikely that the proteins/cytokines recovered may be consequent to local tissue damage caused by probe insertion. The levels of total protein in cerebral dialysate of ~100 μg/mL were 3-fold lower than those found in dialysis fluid from the skin using the same membrane. This is consistent with the maintenance of the blood-brain barrier. However, the finding that ~70% of the total protein that was recovered in cerebral dialysate was albumin is in agreement with similar observations in the skin.

Maurer et al[18] have investigated patterns of global protein expression in cerebral dialysate from patients with stroke using a proteomic approach based on 2D gel electrophoresis and subsequent mass spectrometry. They mapped over 158 protein spots in the 2D gels, of which they identified more than half. The 95 spots identified by subsequent mass spectrometry and database search represented 27 individual proteins, 10 of which were exclusive to the dialysate and not present in cerebrospinal fluid. Common to both were albumin, immunoglobulin (IgG) light and heavy chain, and various binding proteins. Specific to the dialysate were proteins that were brain-specific, such as the creatinine kinase B subunit and tubulin alpha-1, and others that might indicate disease severity or progression.

Of significance in both this study[18] and that of Winter et al[16] is that raised levels of proteins and/or cytokines were detected far from the cerebral injury. It is unlikely that these molecules diffuse freely through the brain[59] and thence that the proteins recovered in the contralateral hemisphere originate from the damaged brain area. It seems more likely that the increases seen are indicative of altered pathology in the undamaged hemisphere and that they provide a biochemical marker of an endogenous response to a secondary diffuse-type damage occurring in the brain and may be important in determining clinical outcome. We do not have comparable values for parenchymal protein or cytokine levels in non–head injured controls, as it is currently not possible to sample dialysate in a "normal" brain, and so we cannot judge the effect that probe insertion itself has on cytokine levels.

Conclusion

In summary, while the high-MWCO membranes are harder to use than those with small pores and the recovery more subject to changes in the local environment, they do provide the opportunity to recover larger bioactive molecules and protein-bound substrates that could otherwise be recovered in vivo. They allow monitoring of changes in interstitial levels of these molecules in a wide range of physiological and pathophysiological states. Microdialysis recovery of large molecules also offers the opportunity to identify patterns of protein expression in a variety of tissue spaces and to evaluate clinically useful biomarkers of disease. From all this may develop a better understanding of the disease process and its diagnosis and more targeted approaches to therapy.

References

1. Bito L, Davson H, Levi EM, Murray M, Snider N. The concentration of free amino acids and other electrolytes in cerebrospinal fluid, in vivo dialysate of brain, and blood plasma of the dog. *J Neurochem*. 1966;13:1057-1067.

2. Petersen LJ, Church MK, Skov PS. Histamine is released in the wheal but not the flare following challenge of human skin in vivo: a microdialysis study. *Clin Exp Allergy*. 1997;27:284-295.

3. Andersson T, Wardell K, Anderson C. Human in vivo cutaneous microdialysis: estimation of histamine release in cold urticaria. *Acta Derm Venereol*. 1995;75:343-347.

4. Krogstad AL, Jansson PA, Gisslen P, Lonnroth P. Microdialysis methodology for the measurement of dermal interstitial fluid in humans. *Br J Dermatol*. 1996;134:1005-1012.

5. Kellogg DL, Jr, Crandall CG, Liu Y, Charkoudian N, Johnson JM. Nitric oxide and cutaneous active vasodilation during heat stress in humans. *J Appl Physiol*. 1998;85:824-829.

6. Klede M, Clough G, Lischetzki G, Schmelz M. The effect of the nitric oxide synthase inhibitor N-nitro-L-arginine-methyl ester on neuropeptide-induced vasodilation and protein extravasation in human skin. *J Vasc Res*. 2003;40:105-114.

7. Clough GF. Role of nitric oxide in the regulation of microvascular perfusion in human skin in vivo. *J Physiol*. 1999;516:549-557.

8. Rhodes LE, Belgi G, Parslew R, McLoughlin L, Clough GF, Friedmann PS. Ultraviolet-B-induced erythema is mediated by nitric oxide and prostaglandin E2 in combination. *J Invest Dermatol*. 2001;117:880-885.

9. Sun L, Stenken JA. Improving microdialysis extraction efficiency of lipophilic eicosanoids. *J Pharm Biomed Anal*. 2003;33:1059-1071.

10. Lindberger M, Tomson T, Wallstedt L, Stahle L. Distribution of valproate to subdural cerebrospinal fluid, subcutaneous extracellular fluid, and plasma in humans: a microdialysis study. *Epilepsia*. 2001;42:256-261.

11. Newman JM, Di Maria CA, Rattigan S, Clark MG. Nutritive blood flow affects microdialysis O/I ratio for [(14)C]ethanol and (3)H(2)O in perfused rat hindlimb. *Am J Physiol Heart Circ Physiol*. 2001;281:H2731-H2737.

12. Tegeder I, Muth-Selbach U, Lotsch J, et al. Application of microdialysis for the determination of muscle and subcutaneous tissue concentrations after oral and topical ibuprofen administration. *Clin Pharmacol Ther*. 1999;65:357-368.

13. Iversen HH, Celsing F, Leone AM, Gustafsson LE, Wiklund NP. Nerve-induced release of nitric oxide in the rabbit gastrointestinal tract as measured by in vivo microdialysis. *Br J Pharmacol*. 1997;120:702-706.

14. Schmelz M, Luz O, Averbeck B, Bickel A. Plasma extravasation and neuropeptide release in human skin as measured by intradermal microdialysis. *Neurosci Lett*. 1997;230:117-120.

15. Winter CD, Iannotti F, Pringle AK, Trikkas C, Clough GF, Church MK. A microdialysis method for the recovery of IL-1beta, IL-6 and nerve growth factor from human brain in vivo. *J Neurosci Methods*. 2002;119:45-50.

16. Winter CD, Pringle AK, Clough GF, Church MK. Raised parenchymal interleukin-6 levels correlate with improved outcome after traumatic brain injury. *Brain*. 2004;127:315-320.

17. Dabrosin C. Variability of vascular endothelial growth factor in normal human breast tissue in vivo during the menstrual cycle. *J Clin Endocrinol Metab*. 2003;88:2695-2698.

18. Maurer MH, Berger C, Wolf M, et al. The proteome of human brain microdialysate. *Proteome Sci*. 2003;1:7.

19. Ernberg MM, Alstergren PJ. Microdialysis of neuropeptide Y in human muscle tissue. *J Neurosci Methods*. 2004;132:185-190.

20. Haskins WE, Watson CJ, Cellar NA, Powell DH, Kennedy RT. Discovery and neurochemical screening of peptides in brain extracellular fluid by chemical analysis of in vivo microdialysis samples. *Anal Chem*. 2004;76:5523-5533.

21. Woodroofe MN, Sarna GS, Wadhwa M, et al. Detection of interleukin-1 and interleukin-6 in adult rat brain, following mechanical injury, by in vivo microdialysis: evidence of a role for microglia in cytokine production. *J Neuroimmunol*. 1991;33:227-236.

22. Sjogren F, Svensson C, Anderson C. Technical prerequisites for in vivo microdialysis determination of interleukin-6 in human dermis. *Br J Dermatol*. 2002;146:375-382.

23. Fellows PJ, Noble MR, Clough GF. Effect of perfusion rate on the recovery of albumin by microdialysis. *J Vasc Res*. 2003;40:304.

24. Riese J, Boecker S, Hohenberger W, Klein P, Haupt W. Microdialysis: a new technique to monitor perioperative human peritoneal mediator production. *Surg Infect (Larchmt)*. 2003;4:11-15.

25. Dabrosin C. Technical aspects of microdialysis of human breast. *Scand J Clin Lab Invest*. 2001;61:269-272.

26. Brown SA, Mayberry AJ, Mathy JA, Phillips TM, Klitzman B, Levin LS. The effect of muscle flap transposition to the fracture site on TNFalpha levels during fracture healing. *Plast Reconstr Surg*. 2000;105:991-998.

27. Licht P, Losch A, Dittrich R, Neuwinger J, Siebzehnrubl E, Wildt L. Novel insights into human endometrial paracrinology and embryo-maternal communication by intrauterine microdialysis. *Hum Reprod Update*. 1998;4:532-538.

28. Trickler WJ, Miller DW. Use of osmotic agents in microdialysis studies to improve the recovery of macromolecules. *J Pharm Sci*. 2003;92:1419-1427.

29. Ao X, Sellati TJ, Stenken JA. Enhanced microdialysis relative recovery of inflammatory cytokines using antibody-coated microspheres analyzed by flow cytometry. *Anal Chem*. 2004;76:3777-3784.

30. Schutte RJ, Oshodi SA, Reichert WM. In vitro characterization of microdialysis sampling of macromolecules. *Anal Chem*. 2004;76:6058-6063.

31. Clough GF, Boutsiouki P, Church MK, Michel CC. Effects of blood flow on the in vivo recovery of a small diffusible molecule by microdialysis in human skin. *J Pharmacol Exp Ther*. 2002;302:681-686.

32. Wiig H, Sibley L, DeCarlo M, Renkin EM. Sampling interstitial fluid from rat skeletal muscles by intermuscular wicks. *Am J Physiol*. 1991;261:H155-H165.

33. Haaverstad R, Romslo I, Larsen S, Myhre HO. Protein concentration of subcutaneous interstitial fluid in the human leg: a comparison between the wick technique and the blister suction technique. *Int J Microcirc Clin Exp*. 1996;16:111-117.

34. Rutili G, Arfors KE. Protein concentration in interstitial and lymphatic fluids from the subcutaneous tissue. *Acta Physiol Scand*. 1977;99:1-8.

35. Hamrin K, Rosdahl H, Ungerstedt U, Henriksson J. Microdialysis in human skeletal muscle: effects of adding a colloid to the perfusate. *J Appl Physiol*. 2002;92:385-393.

36. Sun H, Bungay PM, Elmquist WF. Effect of capillary efflux transport inhibition on the determination of probe recovery during in vivo microdialysis in the brain. *J Pharmacol Exp Ther*. 2001;297:991-1000.

37. Church MK, Griffiths TJ, Jeffery S, et al. Are cysteinyl leukotrienes involved in allergic responses in human skin? *Clin Exp Allergy*. 2002;32:1013-1019.

38. Winter CD, Iannotti F, Pringle AK, Trikkas C, Clough GF, Church MK. A microdialysis method for the recovery of IL-1beta, IL-6 and nerve growth factor from human brain in vivo. *J Neurosci Methods*. 2002;119:45-50.

39. Ao X, Stenken JA. Water-soluble cyclodextrin polymers for enhanced relative recovery of hydrophobic analytes during microdialysis sampling. *Analyst*. 2003;128:1143-1149.

40. Kjellstrom S, Emneus J, Laurell T, Heintz L, Marko-Varga G. On-line coupling of microdialysis sampling with liquid chromatography for the determination of peptide and non-peptide leukotrienes. *J Chromatogr A*. 1998;823:489-496.

41. Davies MI, Cooper JD, Desmond SS, Lunte CE, Lunte SM. Analytical considerations for microdialysis sampling. *Adv Drug Deliv Rev*. 2000;45:169-188.

42. Lunte SM, Lunte CE. Microdialysis sampling for pharmacological studies: HPLC and CE analysis. *Adv Chromatogr*. 1996;36:383-432.

43. Graham DR, Elliott ST, Van Eyk JE. Broad-based proteomic strategies: a practical guide to proteomics and functional screening. *J Physiol*. 2005;563:1-9.

44. Kellar KL, Douglass JP. Multiplexed microsphere-based flow cytometric immunoassays for human cytokines. *J Immunol Methods*. 2003;279:277-285.

45. Anderson L. Candidate-based proteomics in the search for biomarkers of cardiovascular disease. *J Physiol*. 2005;563:23-60.

46. Rohlff C, Hollis K. Modern proteomic strategies in the study of complex neuropsychiatric disorders. *Biol Psychiatry*. 2003;53:847-853.

47. O'Riordan E, Orlova TN, Mei JJ, et al. Bioinformatic analysis of the urine proteome of acute allograft rejection. *J Am Soc Nephrol*. 2004;15:3240-3248.

48. Richter R, Schulz-Knappe P, Schrader M, et al. Composition of the peptide fraction in human blood plasma: database of circulating human peptides. *J Chromatogr B Biomed Sci Appl*. 1999;726:25-35.

49. Tolson J, Bogumil R, Brunst E, et al. Serum protein profiling by SELDI mass spectrometry: detection of multiple variants of serum amyloid alpha in renal cancer patients. *Lab Invest*. 2004;84:845-856.

50. Schmid P. Quantitation of specific proteins in blister fluid. *J Invest Dermatol*. 1970;55:244-248.

51. Nedrebo T, Reed RK, Jonsson R, Berg A, Wiig H. Differential cytokine response in interstitial fluid in skin and serum during experimental inflammation in rats. *J Physiol*. 2004;556:193-202.

52. Iversen VV, Bronstad A, Gjerde EA, Reed RK. Continuous measurements of plasma protein extravasation with microdialysis after various inflammatory challenges in rat and mouse skin. *Am J Physiol Heart Circ Physiol*. 2004;286:H108-H112.

53. Schmelz M, Petersen LJ. Neurogenic inflammation in human and rodent skin. *News Physiol Sci*. 2001;16:33-37.

54. Weidner C, Klede M, Rukwied R, et al. Acute effects of substance P and calcitonin gene-related peptide in human skin: a microdialysis study. *J Invest Dermatol*. 2000;115:1015-1020.

55. Krogstad AL, Lonnroth P, Larson G, Wallin BG. Increased interstitial histamine concentration in the psoriatic plaque. *J Invest Dermatol*. 1997;109:632-635.

56. Garvin S, Dabrosin C. Tamoxifen inhibits secretion of vascular endothelial growth factor in breast cancer in vivo. *Cancer Res*. 2003;63:8742-8748.

57. Simonsen L, Holstein P, Larsen K, Bulow J. Glucose metabolism in chronic diabetic foot ulcers measured in vivo using microdialysis. *Clin Physiol*. 1998;18:355-359.

58. Clough GF, Noble MR. Microdialysis: a model for studying chronic wounds. *Int J Low Extrem Wounds*. 2003;2:233-239.

59. Westerink BH, De Vries JB. A method to evaluate the diffusion rate of drugs from a microdialysis probe through brain tissue. *J Neurosci Methods*. 2001;109:53-58.