Published in final edited form as:

Anal Chem. 2006 November 15; 78(22): 7778–7784.

Microdialysis Sampling Extraction Efficiency of 2-Deoxyglucose: The Role of Macrophages *in vitro* and *in vivo*

Xiaodun Mou and Julie A. Stenken*

Department of Chemistry and Chemical Biology, Center for Biotechnology and Interdisciplinary Studies Rensselaer Polytechnic Institute 110 Eighth Street, Troy, NY, 12180–3590

Abstract

Macrophages are a class of inflammatory cells believed to direct the outcome of device biocompatibility. Despite their relevance to implanted *in vivo* devices, particularly implanted glucose sensors, few studies have attempted to elucidate how these cells affect device performance. Microdialysis sampling probes were used to determine glucose uptake alterations in the presence of resting and activated macrophages *in vitro*. Significant differences for 2-DG relative recovery at 1.0 $\mu\text{L}/\text{min}$ were observed between resting ($74 \pm 7\%$, $n=18$) and lipopolysaccharide (LPS) ($1 \mu\text{g}/\text{mL}$) activated ($56 \pm 6\%$, $n=18$) macrophages in culture which had 2-DG spiked into the media ($p<0.005$). To establish if *in vitro* characterization could be correlated to *in vivo* studies, microdialysis probes were implanted into the dorsal subcutis of male Sprague-Dawley rats for 0,3,5 and 7 days. An internal standard, 2-deoxyglucose (2-DG), was passed through the microdialysis probe during *in vivo* studies. No significant differences in 2-DG extraction efficiency from the probe into the tissue site were observed *in vivo* among microdialysis probes implanted into the subcutaneous space of Sprague-Dawley rats for either three, five or seven days vs. probes implanted the day of sample collection. These results suggest that macrophage activation *in vivo* at implant sites is much lower than highly activated macrophages *in vitro*. It is important to note that these results do not rule out the potential for increased glucose metabolism at sensor implant sites.

Introduction

The creation of reliable implantable glucose sensors for continuous glucose measurements in diabetic humans has been an active research topic for more than three decades.¹ Extensive research efforts have focused on solving many of the challenging problems associated with *in vivo* glucose measurements.^{2,3} Some problems such as removal of electrochemical interferences (e.g., acetaminophen, ascorbate) have been overcome with appropriate electrode design.^{4,5} Other concerns such as direct long-term integration with the host have not been completely solved.⁶⁻⁹

The primary challenge affecting subcutaneous implanted glucose sensors as well as any implanted sensing device has been overcoming the host foreign body response while retaining proper function, e.g., resistance to signal drift and calibration maintenance.^{10,11} The immense tissue-integration challenges associated with *in vivo* glucose measurements have precluded the long-term implantation (1–4 weeks) of glucose sensors for continuous glucose monitoring.¹²⁻¹⁴ Device implantation creates a wound resulting in an activation of complex biochemical and cellular mechanisms to heal the injury, which is referred to as the foreign body response (Figure 1).^{15,16} Research aimed to improve the integration of sensors with their host and thus

* Corresponding Author: Phone: 518–276–2045 Fax: 518–276–4887 Email: stenkj@rpi.edu.

control the foreign body response has focused on chemical release strategies for nitric oxide and vascular endothelial growth factor (VEGF).^{17,18}

Among the different foreign body response mechanisms, fibrotic capsule formation and macrophage recruitment are among the most likely to cause variations in glucose sensor performance (e.g., result in lag times, differences in day-to-day sensitivity, and sensor drift) as denoted in Figure 1.¹⁹⁻²¹ The fibrotic encapsulation around the sensor causes response delays and decreased signal due to analyte diffusional lag through the capsule to the sensor. Macrophage activation at the site of an implanted glucose sensors is poorly understood due to the lack of analytical methods to locally interrogate these cells. Yet, the importance of macrophages towards orchestrating the overall foreign body response has been well documented in the biomaterials literature for twenty years.²²⁻²⁴ Macrophages help to remodel the wound site via release of different chemical signaling agents (cytokines) and enzymes (e.g., various matrix metalloproteinases) and can remain at the implant site throughout its lifetime.

Microdialysis sampling is a diffusion-based separation process that has been widely used for sampling from complex matrices *in vitro* and *in vivo*.²⁵⁻²⁷ The calibration of a microdialysis probe is obtained via its extraction efficiency (*EE*). The steady-state equation for extraction

$$EE = \frac{C_{inlet} - C_{outlet}}{C_{inlet} - C_{sample}} = 1 - \exp\left(\frac{-1}{Q_d(R_d + R_m + R_{ECF} + R_{tr})}\right) \quad \text{Eq.1}$$

efficiency has been derived by Bungay *et al.* and is shown in Eq 1.^{28,29} In this equation, C_{outlet} is the analyte outlet concentration, C_{inlet} is the analyte inlet concentration, and C_{sample} is the analyte concentration far from the probe. When C_{inlet} equals zero, *EE* becomes the ratio of C_{outlet} to C_{sample} , which has been termed relative recovery (*RR*). *EE* is independent of analyte directional movement to or from the dialysis probe and can be used for when analytes are either recovered or delivered through the probe. However, for clarity in this work, all experiments performed in the “recovery” mode where analyte is moving from the sample into the dialysis probe will be ascribed *RR* values. Experiments performed in the “delivery” mode where analyte is infused through the probe and extracted into the sampling media will be denoted as “*EE*.”

Microdialysis sampling *EE* is dependent on flow rate (Q) and diffusional mass transport resistances through the dialysate (R_d), membrane (R_m), tissue space (R_{ECF}), and any trauma layer near the probe (R_{tr}). For any analyte, its *EE* is highly sensitive to the kinetic processes occurring within the sample media external to the microdialysis probe.^{30,31} The mass transport resistance of the tissue is greatly affected by the kinetics of analyte uptake including metabolism (k_m) and capillary permeability, i.e., efflux from ECF to plasma (k_{ep}) and uptake from plasma to ECF (k_{pe}) as denoted in Eqs 2 and 3.^{28,32} In these equations K_0 and K_1 are Bessel functions that typically arise in steady state solutions to diffusion and kinetic mass transfer problems in cylindrical coordinates.¹ The description for is defined as a “combined loss rate constant”, which is a

$$R_{ECF} = \frac{\Gamma [K_0(r_o/\Gamma) / K_1(r_o/\Gamma)]}{2\pi r_o L D_e \phi_e} \quad \text{Eq.2}$$

$$\Gamma = \sqrt{D_e / (k_{ep}^x + k_e^r + k_c^r)} \quad \text{Eq.3}$$

composite function of the analyte diffusion coefficient within the media external to the probe (D_e) and rate constants of the capillary exchange (k_{ep}^x), metabolism of analyte in the ECF (k_e^r), and intra- to extracellular exchange (k_c^r). Besides all the rate constants, the outer membrane

¹Bessel Functions can be evaluated in standard software programs such as Microsoft Excel, Matlab, etc.

radius of the microdialysis probe (r_o), the length of the probe (L), and the volume fraction in the ECF (ϕ_e) also attribute to the mass transport resistance through the tissue space. Knowing that microdialysis sampling extraction efficiency depends on the analyte removal kinetics external to the microdialysis probe as denoted in Eq 3 allows the possibility to test whether or not significant alterations in glucose uptake affect microdialysis *EE* either *in vitro* or *in vivo*.

In this work, the bi-directional diffusive nature of microdialysis sampling was used to determine the effect of glucose uptake kinetic alterations between active and resting macrophages in cell culture as well as *in vivo* on the dialysis probe calibration (*EE* or *RR*) for both glucose and the internal standard 2-deoxyglucose (2-DG) as outlined in Figure 1. 2-DG is routinely used as a tracer of glucose uptake since it is not metabolized by phosphoglucose isomerase (PGI) during glycolysis.³³ Lipopolysaccharide (LPS) was used to stimulate macrophages into an inflammatory state. It has been reported that activated macrophages have greater glucose uptake kinetics when compared to normal tissue cells.³⁴⁻³⁷ If glucose uptake kinetic rates were increased during macrophage activation, this would be reflected as an increase in 2-DG *EE*% (loss from the perfusion fluid) during the microdialysis sampling process. Since activated inflammatory cells are known to be at the site of implants, including sensor implants, changes in glucose uptake kinetics may affect localized glucose sensing.

Experimental Section

Chemicals

Water (HPLC grade, Fisher Scientific, Fair Lawn, NJ) was used to prepare all the solutions. Glucose (Sigma, St. Louis, MO) and 2-deoxyglucose (2-DG, 99%, Acros Organics, NJ) were used as standards for calibration of the microdialysis probes and detection system. All the samples were prepared or diluted in a phosphate buffered saline solution (PBS, pH 7.2 consisting of 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na₂HPO₄, and 1.5 mM KH₂PO₄). Potassium chloride, potassium phosphate monobasic, sodium chloride and sodium phosphate dibasic were all purchased from Fisher Scientific, Fair Lawn, NJ. For macrophage cell culture, the media was the mixture of Dulbecco's Modification of Eagle's Medium (DMEM) (Fisher Scientific, Pittsburgh, PA), fetal bovine serum (FBS, Biowhittaker, Walkersville, MD), and penicillin and streptomycin (Fisher Scientific, Pittsburgh, PA). The DMEM used in general cell culture has a reported glucose concentration of 4.5 g/L (~25 mM). During the microdialysis experiment, DMEM with a glucose concentration of 1.0 g/L was used (~5 mM). Trypan blue solution (0.4%) purchased from Sigma (St. Louis, MO) was used as a dye to count the number of viable cells before and after each cell culture experiment. Bacterial lipopolysaccharide (LPS, E. coli, K-23; Sigma) was employed to induce the macrophages into an inflammatory state. The mobile phase of the detection system contained barium acetate (Ba(OAc)₂, 99%, Fisher Scientific, Pittsburgh, PA) and sodium hydroxide (NaOH) (Mallinckrodt Baker, Inc. Paris, KY, USA). Sterile water (MP Biomedicals, Inc. Solon, OH) was used to perfuse microdialysis probes before implantation *in vivo*.

Ion-exchange Chromatography with Pulsed Amperometric Detection (IC-PAD)

Glucose and 2-deoxyglucose in microdialysates and the cell culture media were directly separated by anion-exchange chromatography followed by quantification with pulsed amperometric detection (IC-PAD).³⁸⁻⁴⁰ The IC-PAD system consisted of an LC-10AD pump (Shimadzu) and an amperometric detector from ANTEC Leyden (DECADE, Antec Leyden, The Netherlands) that contains an internal column heater. An anion-exchange column, Dionex CarboPac PA1, 250 × 2 mm, with guard column (50 × 2 mm) was used for carbohydrate separation. The flow-through detection cell (Antec Leyden VT-03) contained a gold working electrode and Ag/AgCl reference electrode. Pulsed amperometric detection was carried out

with the following pulse potentials and time durations: $E_{\text{DET}} = +0.10 \text{ V}$ ($t_{\text{DET}} = 600 \text{ ms}$), $E_{\text{OX}} = 0.80 \text{ V}$ ($t_{\text{OX}} = 200 \text{ ms}$), $E_{\text{RED}} = -0.60 \text{ V}$ ($t_{\text{RED}} = 200 \text{ ms}$). The PAD detector and columns were maintained at 30°C in an internal column oven. Each day, the ion-exchange columns were regenerated for 30 minutes with a 200 mM sodium hydroxide solution. A solution with 10 mM sodium hydroxide and 2 mM barium acetate was used as the mobile phase. The barium acetate was added to the alkaline solution the day before the experiment to remove excess carbonate via the precipitation of the barium carbonate salt.⁴¹ The mixture was filtered through a $0.2 \mu\text{m}$ filter (Whatman International Ltd. Maidstone, England) before use. This approach removes unwanted carbonate, which improves run-to-run retention time reproducibility. The mobile phase was sparged with argon at low flow rates during analyses. All separations were carried out using an isocratic elution with a 0.25 mL/min flow rate. Concentrations in the dialysates were predicted by comparing peak area to standards of 2-DG and glucose between the range of 0 and $100 \mu\text{M}$ in PBS. A calibration curve was performed on each working day. The microdialysates were diluted 100 times with PBS solution, then separated and analyzed using the IC-PAD system with the same parameters as those for the standards.

Cell Culture

RAW 264.7 murine macrophage cells were obtained as a gift from Professor Michelle Lennartz of Albany Medical College. The culture media (90 v/v% DMEM and 10 v/v% FBS) was supplemented with penicillin (1000 U/mL) and streptomycin ($100 \mu\text{g/mL}$). The macrophage cells were incubated at 37°C with 5% CO_2 using standard cell culture methods.

Macrophages ($1 \times 10^6/\text{mL}$) placed in 2.8 mL cell culture media in the well of a 24-well plate (Tissue culture treated, Corning, NY, USA) were stabilized overnight in DMEM (25 mM glucose, 10% FBS) to allow for cell attachment to the culture plate. On the day of sample collection, fresh media was added to used to replace the overnight media in each well.

Microdialysis System

A BAS Bee microdialysis pump with a 3-syringe bracket and 1-mL syringes (Bioanalytical System Inc., West Lafayette, IN) was used for all microdialysis experiments. Commercially available CMA/20 microdialysis probes consisting of a polycarbonate/polyether (PC) membrane, 10 mm length and 20-kDa molecular weight cutoff (MWCO) (CMA Microdialysis, Inc., North Chelmsford, MA) were immersed into the culture wells containing previously described designed tops.⁴² Unless otherwise stated, a PBS solution was used as the perfusion fluid and was infused with a $1.0 \mu\text{L/min}$ flow rate. All the experiments with microdialysis probes in cell culture media were performed in a cell culture incubator at 37°C with 5% CO_2 . The dialysates were collected into $200\text{-}\mu\text{L}$ microcentrifuge tubes sealed with parafilm to prevent solvent evaporation. The solvent loss during the sample collection period was determined to be $0.3 \pm 0.1 \%$ ($n=10$) comparing the weight of the dialysates with the sample media of the same volume.

Lipopolysaccharide Affect on Glucose Concentrations in Macrophage Culture

The macrophages ($1 \times 10^6/\text{mL}$) in DMEM (10% FBS) with a reported glucose concentration of 25 mM were placed in 6 wells of a 24-well cell culture plate. Serial doses of LPS (1 ng/mL, 10 ng/mL, 100 ng/mL, $1 \mu\text{g/mL}$ and $10 \mu\text{g/mL}$) were added into wells B, C, D, E, and F, separately. The well denoted "A" was the control and contained no LPS. Aliquots ($50 \mu\text{L}$) were taken from each well immediately after LPS addition and at 4, 8, 20 and 24 hours. Each aliquot was mixed with an equal volume of 10 (v/v %) perchloric acid and placed in the refrigerator at 4°C for 10 minutes. The mixture was centrifuged and diluted with PBS by 250 fold. The diluted samples were analyzed using the IC-PAD system.

Alteration of 2-Deoxyglucose Microdialysis *EE* by Macrophages

During each microdialysis sampling experiment, one culture well contained macrophages that served as a control (no LPS) and the other well contained macrophages to which LPS was added. To each well, 2-DG (5 mM) was added to the media containing 5 mM glucose. Microdialysis sampling procedures commenced one hour after the LPS addition and samples were collected every 30 minutes for 3 hours. During the microdialysis sampling process, the concentrations of 2-DG and glucose in sample media were obtained by directly analyzing an aliquot (50 μ L) from the media. After the experiments, the average density of the viable macrophages in the LPS activated wells was $0.95 \pm 0.16 \times 10^6$ cells/mL ($n=3$) as determined by Trypan Blue staining.

In vivo experiments

Male Sprague-Dawley rats (250–300 g, Taconic, Taconic, NY) were used for all *in vivo* experiments. The rats had free access to food and water with a 12-hour on/off light cycle prior to the experiments. The rats were anesthetized with isoflurane and kept warm using a temperature controlled heating pad (CMA Microdialysis, North Chelmsford, MA) during surgery and sample collection. All surgical procedures were performed with aseptic technique. The Albany Medical College IACUC committee has approved these procedures. Prior to implantation, the probes were removed from their sealed packages and perfused with sterile water in 70% ethanol in a biosafety cabinet to remove the glycerol that impregnates the membrane to keep it viable during shipping.

For acute implantations, two 10-mm CMA/20 microdialysis probes with a polycarbonate/polyether (PC) membrane (20 kDa MWCO) were implanted into each side of the dorsal subcutis of the rats. A perfusion fluid containing 2-DG (5 mM in PBS) was infused through both microdialysis probes with a flow rate of 5 μ L/min for five minutes following probe implantation. After this 5-min flushing period, the flow rate was reduced to 1 μ L/min. Following a 15-minute equilibration time at the 1 μ L/min flow rate, the dialysates were then collected every 30 minutes for three hours while the animal was maintained under anesthesia. The dialysates were stored at 4 °C in the refrigerator before dilution and analysis. All samples were analyzed no later than 48 hours after sample collection.

For chronic implantations, one PC probe was implanted on the right side of the dorsal subcutaneous space of rats for up to 3, 5 or 7 days. On the day of dialysate collection, a second PC probe was implanted into the left side of the dorsal subcutaneous space. The remainder of the experimental protocol for sample collection and analysis follows that for the acute implantation procedure described above.

Results and Discussion

Separation of Glucose and 2-DG

To determine retention time reproducibility, glucose and 2-deoxyglucose solutions (25 μ M) were prepared with PBS then separated and analyzed by the ion chromatography-pulsed amperometric (IC-PAD) system. Sample injections (10 μ L) were performed every 15 min over a 6-hour period. The retention times were 5.27 ± 0.10 and 3.76 ± 0.05 minutes ($n=24$) for glucose and 2-DG, respectively. The k' of glucose was 2.0 ± 0.05 , and that of 2-DG was 1.2 ± 0.02 ($n=24$), which illustrates the retention reproducibility for these IC-PAD conditions.⁴³

Microdialysis Sampling of Glucose and 2-Deoxyglucose in Different Sample Media

Microdialysis probes were tested in different media to determine if protein from the culture media would affect extraction efficiency for either 2-DG or glucose. Table 1 shows the resulting *RR%* values obtained among three separate media (PBS, culture media without fetal bovine

serum (FBS) and culture media with 1% FBS). Among these three different media, *RR%* values were roughly 70% and were not different.

Macrophage activation

A range of LPS concentrations between a blank (no LPS) and 10 $\mu\text{g/mL}$ were applied to the macrophage-containing wells to determine the level at which glucose consumption was significantly increased. Table 2 shows the resulting glucose concentrations measured over a 24-hour period obtained from the individual wells. After 4 hours, there were significant differences between the glucose concentrations in wells with LPS as compared to the blank. Macrophage cultures with either 1 or 10 ng/mL LPS exhibited similar glucose concentrations ($\sim 18 \text{ mM}$) at 4 hours. Glucose concentrations obtained from macrophages given the doses ranging from 100 ng/mL to 10 $\mu\text{g/mL}$ were less than those obtained for the 1 and 10 ng/mL dose. Based on these experiments, a concentration of 1 $\mu\text{g/mL}$ of LPS was chosen for the remaining cell culture experiments.

2-Deoxyglucose and Glucose *RR* in Macrophage Cell Culture

Microdialysis sampling recovery (*RR%*) values for 2-DG and glucose were significantly different between resting and LPS-activated macrophages (Table 3). Table 3 shows the individual statistics for glucose and 2-DG microdialysis sampling *RR%* and concentration values obtained from aliquots of the culture media at 30-minute increments within the macrophage culture wells. The overall average among all the data points showed significant decreases in 2-DG ($56 \pm 6 \%$) and glucose ($61 \pm 9 \%$) *RR%* with the active macrophages compared with those (2-DG, $74 \pm 7 \%$; glucose, $74 \pm 7 \%$) with resting macrophages. For 2-DG, a clear reduction in *RR%* in the activated vs. resting macrophages was evident at the 95% confidence level for three of the six time points and was at least at the 85% confidence level for all sampling periods. The glucose microdialysis sampling *RR%* in the culture wells was also significantly different between the resting and activated macrophages at the 90% confidence level or greater for four of the six time points. The cause of the large variation in the glucose concentrations and thus microdialysis *EE%* at the 60- and 120-minute collection period is uncertain.

2-Deoxyglucose and Glucose *EE* in Macrophage Cell Culture

Prior to *in vivo* studies, microdialysis probes were placed into macrophage cultures with LPS addition and perfused with 2-DG in a delivery mode. Figure 2 depicts the 2-DG *EE%* and glucose *RR%* between the resting and LPS-activated macrophages as a function of time. The 2-DG average *EE%* obtained from the LPS-activated macrophages ($63 \pm 7 \%$) across all the time points is significantly greater than that from resting macrophages ($36 \pm 9 \%$) at the 95% confidence level ($p < 0.05$). The change in 2-DG *EE%* reflects the alteration in the localized uptake of the infused 2-DG during the microdialysis sampling process. The reason for the low *EE%* values for 2-DG at 90 and 150 minutes are uncertain and may be due to experimental error with this biological system.

This *in vitro* data shows that activated macrophages affect microdialysis sampling *EE%*. This difference in microdialysis sampling *EE%* is not possible without a significant increase in the macrophage 2-DG uptake kinetics. While it is not possible to measure the glucose concentration at the probe outer radius, an increase in localized glucose uptake near a sensor surface could affect glucose detection. More importantly a localized increase in glucose uptake could affect calibration and lead to measurement error since implanted glucose sensors are calibrated relative to blood values.

2-Deoxyglucose *in vivo* Microdialysis

To further test if 2-DG can be used as an internal standard to determine macrophage activation at the implant site, microdialysis probes were implanted into subcutaneous tissue of male Sprague Dawley rats. In each experimental animal, one probe was implanted for a set period of time (3, 5, and 7 days) on one side of the dorsal subcutis while another probe was acutely implanted as a control on the opposite side on the day of sample collection. The 3-day implantation experiment has been performed using three animals; the 5-day and 7-day implantation experiments were each performed using two animals. Figure 3 illustrates the average 2-DG *EE%* and glucose concentrations obtained from probes implanted for 3 days vs. those implanted the day of sample collection paired within the same animal ($n=3$ animals). The *in vivo* 2-DG *EE%* values and glucose concentrations at 5 and 7 days compared to the control values are presented in Figures 4 and 5, respectively.

Table 4 shows the average 2-DG *EE%*s and glucose concentrations across all the time points for the acute vs. chronic implanted dialysis probes at three, five and seven days. The assumption in these experiments is that a steady state *EE%* was achieved during the microdialysis sampling process. This assumption was confirmed by comparing dialysates obtained after the first 15 minutes of implantation using a 1.0 $\mu\text{L}/\text{min}$ flow rate with those obtained at the first 30-min collection period (after this initial flush period). Among these dialysates there were no differences in the 2-DG *EE%* or the glucose concentration (data not shown). Therefore, the six collected dialysates over the three-hour sampling period for each probe were averaged and compared. Table 4 shows that except for a few outliers the 2-DG *EE%* and glucose concentrations between the acute and chronic implanted probes were not statistically different at the 95% confidence level.

Summary of *In Vitro* and *In Vivo* Data

Macrophages begin to accumulate to implanted surfaces within a few hours and can remain at the site throughout the implant lifetime.¹⁴ Based on this information, glucose uptake could be altered during the early period of the inflammatory response at an implant site. The *in vivo* 2-DG *EE%* values vary significantly compared to those *in vitro*. For the *in vivo* studies, there are few differences in 2-DG *EE%* and glucose consumption between probes implanted acutely vs. those implanted for either 3-, 5-, or 7-days. These observed differences between *in vitro* and *in vivo* 2-DG *EE%* are likely due to the degree of the inflammatory response as well as a potential difference in macrophage cell density per unit volume. The addition of lipopolysaccharide greatly activates cells in culture to an inflammatory state potentially making the *in vitro* cells far more metabolically active than those *in vivo*.

The sensitivity of the dialysis probe towards small external metabolic changes could come into play in these studies. We have previously shown that for an *in vitro* enzyme system that a 20-fold difference in enzymatic activity was required to obtain an statistically significant alteration in observed *EE%* differences for a substrate.⁴⁴ This is an important point for the *in vitro* experiments since statistically significant differences in *RR%* and *EE%* were observed suggesting a very large change in metabolic activity despite the moderate change in the extraction efficiency values. It should be noted that differences in product concentrations collected into microdialysis sampling probes as a function of time are observable for small changes in enzymatic activity external to a probe. However, in these studies, 2-DG is trapped inside the cell. While the phosphorylated metabolite (2-DG-6-phosphate) that accumulates into cells would be a reasonable target *in vitro* for measuring metabolism differences, it is not possible to measure this product *in vivo* since it is not released into the extracellular fluid space.⁴⁵

In addition to activated macrophages, other tissue processes can affect *EE%* during a long-term microdialysis probe implant and include biofouling of the membrane and tissue mass transport resistance changes caused by the foreign body response.⁴⁶ It is known that for small molecules, the tissue resistance contributes most to the variation in the analyte *EE%*.

It is interesting to note that among the 3, 5 and 7 day implants, there was little difference in the 2-DG *EE%* as compared to the probes implanted as controls. In some cases, the longer term implanted probes exhibited higher delivery than the acute implant. This could potentially be caused by an edema that would create additional wound fluid around the device and would serve to decrease the analyte mass transfer resistance through the tissue (e.g., the volume fraction increases).

Conclusions

The IC-PAD chromatographic method coupled with the macrophage cell line provided a straightforward method to measure glucose uptake alterations near the microdialysis probe. The effects of the foreign body response on analyte collection are an integrated whole that have not previously been systematically studied. This work shows that macrophages *in vivo* appear to be less reactive towards the microdialysis probe than those activated *in vitro*. Additionally, no significant differences in either 2-DG *EE%* or glucose concentrations were observed among microdialysis probes implanted for 3, 5 and 7 days.

Acknowledgments

We thank Dr. Michelle Lennartz and Dr. Daniel Loegering at the Center for Cell Biology and Cancer Research and the Center for Cardiovascular Sciences at the Albany Medical College for their guidance with cell culture methodology and help with some of the animal experiments. We gratefully acknowledge the support from NIH EB001441. The helpful comments from all the manuscript reviewers are also gratefully acknowledged.

References

1. Clark LC, Sachs G. Ann. N.Y. Acad. Sci 1968;148:133–153. [PubMed: 5237638]
2. Wilson GS, Hu Y. Chem. Rev 2000;100:2693–2704. [PubMed: 11749301]
3. Heller A. Ann. Rev. Biomed. Eng 1999;1:153–175. [PubMed: 11701486]
4. Zhang Y, Hu Y, Wilson GS, Moatti-Sirat D, Poitout V, Reach G. Anal. Chem 1994;66:1183–1188. [PubMed: 8160962]
5. Chen T, Friedman KA, Lei L, Heller A. Anal. Chem 2000;72:3757–3763. [PubMed: 10959960]
6. Gifford R, Kehoe JJ, Barnes SL, Kornilayev BA, Alterman MA, Wilson GS. Biomaterials 2006;27:2587–2598. [PubMed: 16364432]
7. Wilson GS, Gifford R. Biosens. Bioelectron 2005;20:2388–2403. [PubMed: 15854814]
8. Long N, Yu B, Moussy Y, Moussy F. Diabetes Technol. Ther 2005;7:927–936. [PubMed: 16386099]
9. Ahmed S, Dack C, Farace G, Rigby G, Vadgama P. Anal. Chim. Acta 2005;537:153–161.
10. Wisniewski N, Moussy F, Reichert WM. Fresen. J. Anal. Chem 2000;366:611–621.
11. Ratner BD. J. Controlled Release 2002;78:211–218.
12. Gilligan BJ, Shults MC, Rhodes RK, Jacobs PG, Brauker JH, Pintar TJ, Updike SJ. Diabetes Technol. Ther 2004;6:378–386. [PubMed: 15198842]
13. Pickup JC, Hussain F, Evans ND, Sachedina N. Biosens. Bioelectron 2005;20:1897–1902. [PubMed: 15741056]
14. Choleau C, Klein JC, Reach G, Aussedat B, Demaria-Pesce V, Wilson GS, Gifford R, Ward WK. Biosens. Bioelectron 2002;17:641–646. [PubMed: 12052349]
15. Anderson JM. Annu. Rev. Mater. Res 2001;31:81–110.
16. Koenig, AL.; Grainger, DW. Biomimetic Materials and Design. Dillow, AK.; Lowman, AM., editors. Marcel Dekker, Inc; New York: 2002. p. 187–250.

17. Gifford R, Batchelor MM, Lee Y, Gokulrangan G, Meyerhoff ME, Wilson GS. *J. Biomed. Mater. Res* 2005;75A:755–766.
18. Ward WK, Wood MD, Casey HM, Quinn MJ, Federiuk IF. *Diabetes Technol. Ther* 2004;6:137–145. [PubMed: 15117580]
19. Woodward SC. *Diabetes Care* 1982;5:278–281. [PubMed: 7172997]
20. Clark LC, Spokane RB, Homan MM, Sudan R, Miller M. *ASAIOT Transactions* 1988;34:259–265. [PubMed: 3196517]
21. Rebrin K, Steil GM. *Diabetes Technol. Ther* 2000;2:461–472. [PubMed: 11467349]
22. Anderson JM, Miller KM. *Biomaterials* 1984;5:5–10. [PubMed: 6375747]
23. Rhodes NP, Hunt JA, Williams DF. *J. Biomed Mater Res* 1997;37:481–488. [PubMed: 9407296]
24. Labow RS, Meek E, Santerre JP. *Biomaterials* 2001;22:3025–3033. [PubMed: 11575477]
25. Weintjes KJ, Vonk P, Vonk-Van Klein Y, Schoonen AJM, Kossen NW. *Diabetes Care* 1998;21:1481–1488. [PubMed: 9727895]
26. Hillered L, Persson L. *Scandinavian Cardiovascular Journal* 2003;37:13–17. [PubMed: 12745797]
27. de la Pena A, Liu P, Derendorf H. *Adv. Drug Delivery Rev* 2000;45:189–216.
28. Bungay PM, Morrison PF, Dedrick RL. *Life Sci* 1990;46:105–19. [PubMed: 2299972]
29. Bungay PM, Newton-Vinson P, Isele W, Garriss PA, Justice JB. *J. Neurochem* 2003;86:932–946. [PubMed: 12887691]
30. Robinson, TE.; Justice, JB., editors. *Microdialysis in the Neurosciences*. Elsevier; Amsterdam: 1991.
31. Stenken JA, Lunte CE, Southard MZ, Stähle L. *J. Pharm. Sci* 1997;86:958–966. [PubMed: 9269875]
32. Stenken JA. *Anal. Chim. Acta* 1999;379:337–357.
33. Wick AN, Drury DR, Nakada HI, Wolfe JB. *J. Biol. Chem* 1957;224:963–969. [PubMed: 13405925]
34. Hamilton JA, Vairo G, Lingelbach SR. *J. Cell. Physiol* 1988;134:405–412. [PubMed: 2832422]
35. Meszaros K, Lang CH, Bagby GJ, Spitzer JJ. *FASEB J* 1988;2:3083–3086. [PubMed: 3056766]
36. Lang CH, Dobrescu C. *Metab. Clin. Exp* 1991;40:585–593.
37. Fernandes LC, Mattozo CA, Machado UF, Costa Rosa LFBP, Curi R. *Cell Biochem. Funct* 1996;14:187–192. [PubMed: 8888572]
38. Rakotomanga S, Baillet A, Pellerin F, Baylocq-Ferrier D. *J. Pharm. Biomed. Anal* 1992;10:587–591. [PubMed: 1463793]
39. Johnson DC, LaCourse WR. *Anal. Chem* 1990;62:589A–597A.
40. Zook CM, LaCourse WR. *Anal. Chem* 1998;70:801–806.
41. Cataldi T, Campa C, Angelotti M, Bufo SA. *J. Chromatogr. B* 1999;855:539–550.
42. Sun L, Stenken JA. *J. Chromatogr. B* 2003;796:327–338.
43. Andreas G, Andreas S. *Microchim. Acta* 2004;146:97–102.
44. Steuerwald AJ, Villeneuve JD, Sun L, Stenken JA. *J. Pharm. Biomed. Anal* 2006;40:1041–1047. [PubMed: 16242888]
45. Sasson S, Oron R, Cerasi E. *Anal. Biochem* 1993;215:309–311. [PubMed: 8122797]
46. Wisniewski N, Klitzman B, Miller B, Reichert M. *J. Biomed. Mater. Res* 2001;57:513–21. [PubMed: 11553881]

Figure 1.

The foreign body response progression with the role of the microdialysis probe in this study.

Figure 2.

Localized infusion (microdialysis delivery) of 5 mM 2-DG at 1.0 $\mu\text{L}/\text{min}$ through the microdialysis probe to macrophage cultures to which LPS has been added (■) or resting (▲) macrophages. The culture media initially contains 5 mM glucose. The term *EE%* denotes the amount of 2-DG extracted from the infusion into the macrophage culture wells.

Figure 3. 2-DG EE% (left) and glucose concentrations (mM) (right) collected from dialysates from acute (■) and 3-day (▲) implanted PC probes placed into the subcutaneous space of anesthetized rats (n=3). The probes were infused with 5 mM 2-DG at 1.0 μ L/min. Symbols and error bars denote means $\pm$ S.D.

Figure 4. 2-DG EE% (left) and glucose concentrations (mM) (right) collected from dialysates from acute (Day 0) (■, □) and 5-day (▲, △) PC probes implanted into the subcutaneous space of anesthetized rats (n=2). The probes were infused with 5 mM 2-DG at 1.0 μ L/min. Individual symbols denote values from each animal.

Figure 5. 2-DG EE% (left) and glucose concentrations (mM) (right) collected from dialysates from acute (Day 0) (■,□) and 7-day (▲,△) PC probes implanted into the subcutaneous space of anesthetized rats (n=2). The probes were infused with 5 mM 2-DG at 1.0 μ L/min. Individual symbols denote values from each animal.

Table 12-Deoxyglucose and glucose microdialysis sampling *RR*%

Sample Media	2-DG	Glucose	Probe "n"	Sample "n"
PBS	70±5	71±5	3	4
Culture Media without FBS	76±6	75±4	3	4
Culture Media (1% FBS)	78±9	74±5	3	4

Microdialysis sampling was performed at 1.0 μ L/min with PBS buffer at 37°C. All media contained 5 mM 2-DG and glucose. Samples were collected for 30 minutes over a 2-hour period. Data represent mean $\pm$ S.D averaged among all samples collected in each type of media (n=12).

Table 2

Glucose concentration (mM) after lipopolysaccharide addition to cultured macrophages.

LPS Dose	0	4 hr	8 hr	20 hr	24 hr
Blank	23.1	21.5	15.8	15.3	14.6
1 ng/mL	23.0	17.5	17.4	15.4	14.0
10 ng/mL	22.8	18.0	16.4	14.0	9.9
100 ng/mL	22.5	15.3	13.9	10.4	8.6
1 µg/mL	22.8	13.9	14.9	14.2	9.4
10 µg/mL	22.6	14.3	13.1	13.1	8.6

Macrophages (1×10^6 /mL) in DMEM culture media with a reported glucose concentration of 4.5 g/L (25 mM) were placed into individual wells of a 24-well cell culture plate. Individual aliquots (50 µL) were removed and analyzed at the specified time periods.

Table 3
Relative recovery (RR%) and concentrations of 2-deoxyglucose and glucose in macrophage cultures.

2-Deoxyglucose						
Time (min)	30	60	90	120	150	180
RR% LPS	51±5	61±6	54±10	56±8	56±3	56±5
RR% Resting	75±7	73±3	78±9	72±7	75±9	70±10
<i>p</i> (<i>t</i> -test) RR%	0.03 [‡]	0.12	0.10	0.10	0.04 [‡]	0.05 [‡]
[2-DG] _{well} LPS, mM	5.2±0.2	5.1±0.1	5.1±0.2	4.9±0.1	5.0±0.1	4.8±0.1
[2-DG] _{well} Resting, mM	4.9±0.2	4.9±0.2	4.9±0.2	4.9±0.1	4.8±0.2	4.8±0.2
Glucose						
Time (min)	30	60	90	120	150	180
RR% LPS	57±1	66±9	60±13	64±15	58±5	62±10
RR% Resting	72±3	74±3	79±6	73±11	74±9	73±11
<i>p</i> (<i>t</i> -test) RR%	0.02 [‡]	0.30	0.09	0.32	0.02 [‡]	0.01 [‡]
[Glucose] _{well} LPS	5.2±0.3	5.2±0.2	5.2±0.2	5.0±0.2	4.9±0.2	4.6±0.3
[Glucose] _{well} Resting	5.2±0.0	5.2±0.0	5.1±0.0	5.1±0.1	5.0±0.1	5.0±0.1

Time denotes the time at which a sample was obtained from the microdialysis probes after adding LPS (1 µg/mL) to the culture wells (n=3). Microdialysis probes are directly immersed in the well containing both media and macrophages and perfused at 1.0 µL/min. Measurements of 2-DG and glucose concentrations from the wells were performed by removing media which bathes the macrophages that are attached to the culture plate. Data represent mean ± S.D.

[‡], denotes a statistically significant difference between the microdialysis RR% from resting as compared to LPS-activated macrophages at the 95% confidence level.

Table 4*In vivo* 2-DG delivery (*EE*%) and glucose concentrations.

Rats	<i>EE</i> % of 2-DG		Glucose Concentration [mM]	
	Acute	Day 3	Acute	Day 3
3-Day Implantation	38±6	45±3	5.5±0.4	6.6±0.6 [†]
	32±8	41±5 [†]	5.4±1.1	5.6±0.9
	32±7	39±10	4.7±0.7	4.2±0.5
5-Day Implantation	Acute	Day 5	Acute	Day 5
	45±4	44±4	3.9±1.0	3.2±0.3
	29±4	40±4 [†]	5.8±1.0	4.8±1.4 [†]
7-Day Implantation	Acute	Day 7	Acute	Day 7
	41±6	36±4 [†]	5.3±1.3	4.6±0.3
	31±10	34±8	6.4±1.0	5.6±0.6

Probes were implanted into the subcutaneous space and infused with 5 mM 2-DG at 1.0 μ L/min over a 3-hr period with samples collected every 30 minutes while the animal was maintained under anesthesia. *EE*% denotes percent 2-DG extracted from the probe into the tissue and was averaged over the six samples obtained during the 3-hr period. Data represent mean $\pm$ S.D, n=6.

[†], denotes a statistically significant difference between the acute and chronic implant 2-DG microdialysis *EE*% at the 95% confidence level.