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SYMPOSIUM

Modulation of the Foreign Body Reaction for Implants in the Subcutaneous Space: Microdialysis Probes as Localized Drug Delivery/Sampling Devices

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Abstract

Modulation of the foreign body reaction is considered to be an important step toward creation of implanted sensors with reliable long-term performance. In this work, microdialysis probes were implanted into the subcutaneous space of Sprague-Dawley rats. The probe performance was evaluated by comparing collected endogenous glucose concentrations with internal standard calibration (2-deoxyglucose, antipyrine, and vitamin B_{12}). Probes were tested until failure, which for this work was defined as loss of fluid flow. In order to determine the effect of fibrous capsule formation on probe function, monocyte chemoattractant protein-1/CC chemokine ligand 2 (MCP-1/CCL2) was delivered locally via the probe to increase capsule thickness and dexamethasone 21-phosphate was delivered to reduce capsule thickness. Probes delivering MCP-1 had a capsule that was twice the thickness (500–600 μm) of control probes (200–225 μm) and typically failed 2 days earlier than control probes. Probes delivering dexamethasone 21-phosphate had more fragile capsules and the probes typically failed 2 days later than controls. Unexpectedly, extraction efficiency and collected glucose concentrations exhibited minor differences between groups. This is an interesting result in that the foreign body capsule formation was related to the duration of probe function but did not consistently relate to probe calibration.

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Introduction

Continuous *in vivo* sampling or sensing of different endogenous compounds directly in tissue is of significant biomedical importance.[1–3] There is significant research interest in sensors that are implanted subcutaneously that measure glucose due to the clinically important need to monitor glucose in persons with diabetes.[4–6] The achievement of reliable long-term implantable glucose sensors that can be used accurately for more than a week

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Abbreviations: (2-DG) 2-deoxyglucose, (ANOVA) analysis of variance, (BSA) bovine serum albumin, (dex) dexamethasone, (dex-phos) dexamethasone 21-phosphate, (*EE*) extraction efficiency, (ESI) electron spray ionization, (HPLC) high-performance liquid chromatography, (IC-PAD) ion-exchange chromatography with pulsed amperometric detection, (MCP-1/CCL2) monocyte chemoattractant protein-1/CC chemokine ligand 2, (MWCO) molecular weight cutoff, (MS) mass spectroscopy, (PBS) phosphate-buffered saline, (PES) polyethersulfone, (UV) ultraviolet, (VB_{12}) vitamin B_{12}

Keywords: calibration, dexamethasone 21-phosphate, foreign body reaction, glucose, in vivo microdialysis sampling, MCP-1/CCL2

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has been impeded due to the foreign body reaction, which affects the sensor performance in the *in vivo* environment.[7,8] Among the different foreign body reaction mechanisms, fibrous capsule formation and the resulting loss of solute supply and capillary density during the neovascularization process along with 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.[9,10] Achieving long-term integration with the host, long-term device calibration, and controlling biofouling are still unresolved challenges with implanted glucose sensors.[11–15] While it is known the foreign body reaction plays an important role with respect to long-term integration of a foreign object with its host, it is not fully known what exactly leads to either failure of the sensor or loss of calibration.[9]

Microdialysis sampling has become a well-established method for *in vivo* collection of both endogenous and xenobiotic solutes via diffusion through a semipermeable membrane with defined molecular weight cutoff (MWCO).[16] Microdialysis sampling probes have been implanted into different tissue sites, including but not limited to brain, liver, and skin in experimental animals and humans for real-time collection and delivery of various solutes.[17] The possibility of simultaneous collection of solutes combined with the concomitant delivery of drugs or internal standards to the implant site is unique and certainly cannot be obtained with other sensors or drug delivery devices.

The calibration of the microdialysis probe is obtained through its extraction efficiency [*EE*, **Equation (1)**]. The steady-state equation for *EE* has been derived by Bungay and colleagues.[18,19] 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. 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}) that could include the foreign body capsule or other tissue with different diffusive or kinetic properties as compared to healthy tissue (R_{ECF}) far away from the implant site. The mass transport resistance of the tissue is also greatly affected by both the diffusion of the solute and kinetic components, i.e., metabolism and capillary permeability. The mass transport resistance equations denoted in **Equation (1)** are defined in **Equations (2)** and **(3)**, where D is the diffusion coefficient through the dialysate D_d, membrane D_m, and sample D_s (or *ECF*); L is the membrane length, r_i, r_o, and r_α are the inner membrane, external membrane, and cannula radii, respectively; and Γ (cm) is a composite function consisting of rate constants with Bessel function dependence (K_0 and K_1) as a function of radial position related to capillary permeability $k_{ep}(r)$, metabolism $k_m(r)$, and other terms $k_c(r)$ that have been defined by others.[18,20]

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

$$R_d = \frac{13(r_i - r_\alpha)}{70\pi L r_i D_d};\ R_m = \frac{\ln(r_o / r_i)}{2\pi L D_m \phi_m};\ R_e = \frac{\Gamma[K_0(r_o/\Gamma)/K_1(r_o/\Gamma)]}{2\pi r_o L D_s \phi_s} \quad (2)$$

$$\Gamma = \sqrt{\frac{D_s}{(k_{ep}(r) + k_m(r) + k_c(r))}} \quad (3)$$

The goal of this work was to use the microdialysis sampling probe as a means to deliver MCP-1 (a pro-inflammatory chemokine) or dexamethasone (an anti-inflammatory drug) concomitantly with different internal standards to assess how the foreign body reaction affects the *EE* for probes implanted into the subcutaneous space of Sprague-Dawley rats (**Figure 1**). Rats were used since they are often used for glucose sensor testing,[21,22] microdialysis sampling,[23,24] and different biomaterials implantation studies.[25,26] In this work, the internal standards chosen were 2-deoxyglucose (2-DG), antipyrine, and vitamin B_{12} (VB_{12}), and the logic for these choices has been described previously.[27] These choices were made

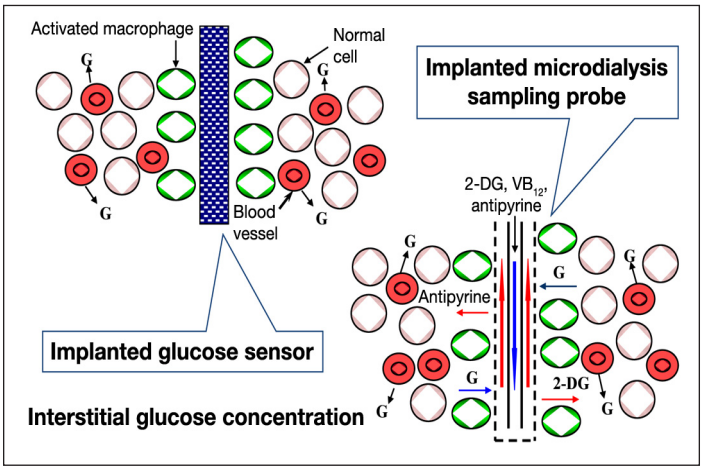

Figure 1. Comparison between implanted glucose sensor and microdialysis probe.

to ascertain if localized metabolism (2-DG), localized blood flow (antipyrine and VB_{12}), and/or biofouling (all three calibrators) of the membrane itself affected the calibration of the sampling probes. Antipyrine and VB_{12} would not be expected to be metabolized to a significant extent in subcutaneous tissue since these two solutes are metabolized in the liver. The use of an awake and freely-moving animal system allowed daily *in vivo* sampling from freely moving animals during the long-term implantation, thus preventing the potential undefined changes that anesthetics may cause. An additional benefit of the awake and freely-moving system is that physiological conditions are more easily mimicked.

We chose to use the internal standards in a retrodialysis (localized delivery) format under the assumption that *EE%* is independent of solute transport direction. While other microdialysis *in vivo* calibration techniques exist, the zero-net flux and approach to zero-flow techniques are highly time-consuming and would not allow the throughput that we desired in this work. Because the internal standards are all xenobiotic compounds, we chose the retrodialysis (delivery of internal standards) technique because of its ease of use in our experimental design.

The *EE* for the different internal standards after localized delivery of different modulating agents compared to controls were determined over a period of 7 to 10 days postimplantation. Monocyte chemoattractant protein-1/CC chemokine ligand 2 (MCP-1/CCL2) was chosen as a positive control since its presence should serve to activate the immune response to the implanted probe as well as direct monocytes to the implant.[28] Dexamethasone 21-phosphate (dex-phos), which is converted to dexamethasone by esterases *in vivo*,[29] was used as an anti-inflammatory agent because it has long been used in biomaterials studies to reduce the impact of the foreign body response.[30,31]

Materials and Methods

Chemicals and Solutions

Antipyrine, bovine serum albumin (BSA), dexamethasone, dex-phos, and VB_{12} were obtained from Sigma-Aldrich (St. Louis, MO); 2-DG was purchased from Acros Organics (Fair Lawn, NJ); monocyte chemoattractant protein-1 (MCP-1) was purchased from BD Biosciences (Franklin Lakes, NJ). All other chemicals were reagent grade or better. **Table 1** shows all the molecular weight and diffusion coefficient data for the different chemicals.

Table 1.
Molecular Characteristics of Solutes

Solute	Molecular weight (Da)	Reported diffusion coefficient (cm^2/s)[a]
2-Deoxyglucose	164	5.3×10^{-6} (25 °C)[b]
Antipyrine	188	6.8×10^{-6} (25 °C)[c]
Dexamethasone	392	$4.01 \pm 2.01 \times 10^{-6}$ [d]
Dex-phos	470	Not evaluated
Glucose	180	6.8×10^{-6} (25 °C)[e]
MCP-1	14,100	0.93 and 1.08×10^{-6} [f]
Vitamin B_{12}	1,355	4.1×10^{-6} [g]

[a] Diffusion coefficients measured in aqueous systems.
[b] Hazel and Sidell[32]
[c] Snyder and colleagues;[33] Wilke-Chang approximation
[d] Moussy and colleagues;[34] measurement *in vivo* in rat subcutaneous tissue
[e] Renkin[35]
[f] Altieri and colleagues[36] and Paavola and colleagues.[37] The human form of MCP-1 is ~8,600 Da. The rat form is 14,100 Da and is a larger protein.
[g] Kanamori and colleagues[38]

Phosphate-buffered saline (PBS) was made by dissolving sodium chloride (NaCl, 137 mM), potassium chloride (KCl, 2.7 mM), sodium phosphate dibasic (Na_2HPO_4, 8.1 mM), and potassium phosphate monobasic (KH_2PO_4, 1.5 mM) in high-performance liquid chromatography (HPLC) grade water (Fisher Scientific, Fair Lawn, NJ). MCP-1 dilutions were prepared in PBS with 0.1 wt% BSA. All solutions were sterile-filtered (0.2 μm) (Corning Inc., Kaiserslautern, Germany) in a biosafety cabinet before use.

Standards and Analytical Detection System

The standards, glucose, VB_{12}, antipyrine, and 2-DG (99%), were dissolved in PBS (pH 7.2) and used to calibrate the detection system and microdialysis probes every day. The dialysates collected during the *in vivo* microdialysis were analyzed using two different HPLC systems. Antipyrine and VB_{12} were measured using reversed-phase chromatography with ultraviolet (UV) detection. Glucose and 2-DG were analyzed by an ion-exchange chromatography with pulsed amperometric detection (IC-PAD) system that has been described elsewhere.[27]

Vitamin B_{12} and Antipyrine

Vitamin B_{12} and antipyrine were separated with an Aqua C18 column (125Å, 150 × 2 mm; Phenomenex, Torrance, CA) using a Shimadzu HPLC system (Columbia, MD) consisting of an SIL-10ADvp autoinjector, a LC-10ADvp pump, a DGU-14A degasser, a CTO-10ASvp column oven, and an

SCL-10Avp system controller with no pretreatment or dilution, and a UV-VIS detector (SPD-10AVvp) with the wavelength set to 245 nm. The mobile phase consisting of 23% (v/v) acetonitrile in 0.05 M phosphate buffer (pH 2.7) was pumped at 0.1 ml/min. Standard solutions consisting of 20, 40, 60, 80, and 100 μM of antipyrine and VB_{12} were prepared daily. The sample injection volume was 10 μl.

Glucose and 2-Deoxyglucose

An ion-exchange chromatography column (CarboPac PA1; Dionex, Sunnyvale, CA) was used for separations followed by pulsed amperometric detection (IC-PAD). An LC-10AD pump (Shimadzu) and an amperometric detector (DECADE, Antec Leyden, The Netherlands) was used for the analysis of glucose and 2-DG and this method has been described previously.[27]

Standards containing glucose and 2-DG (20, 40, 60, 80, and 100 μM) were used to calibrate the IC-PAD system every working day. Dialysate samples were diluted by taking 5 μl of dialysate to which 495 μl of dialysis perfusion fluid was added. From this sample, 10 μl was used for the analysis. Dilution was necessary to have concentrations within the linear range of the electrochemical detector.

MCP-1

MCP-1 was measured with a rat MCP-1 immunoassay kit (optEIA; BD Biosciences, Franklin Lakes, NJ) using a Tecan SpectraFluor plate reader (Tecan Group Ltd., Männedorf, Switzerland) at 450 nm (corrected at Abs 570 nm). Microdialysis samples (25 μl) were diluted to 100 μl with the kit assay diluent to meet the sample volume requirements for this kit.

Dexamethasone 21-Phosphate

Microdialysates obtained from the *in vivo* delivery of dex-phos and the internal standards were quantified using HPLC coupled to an electron spray ionization mass spectroscopy (HPLC-ESI/MS) detection system. The Agilent 1100 series HPLC (Agilent Technologies, Santa Clara, CA) composed of an autosampler, a binary pump, and an Alltima 5 μm C18 column (150 × 4.6 mm) with guard column were used for the sample separation and 5 μl samples were injected. A gradient elution was used with the following conditions: solvent A was 5 mM NH_4OAc (pH 4.5), and solvent B was HPLC grade acetonitrile. During the gradient, solvent B conditions were as follows: 25–30% (v/v), 0–3 min; 30–48%, 3–12 min, 48–70%, 12–17 min; 70%, 17–22 min; 25–70%, 22–25 min; 25%, 25–30 min was applied at a flow rate of 0.5 ml/min and detection was at 245 nm. The MSD 1100 Ion Trap mass analyzer (Agilent Technologies, Santa Clara, CA) operating in the electron spray ionization (ESI) positive/negative ion mode was connected to the HPLC system as the detector. The following mass spectroscopy (MS) conditions were set during the detection: capillary voltage 4.0 kV, endplate offset -500 V, nebulizer 25 psi, dry gas 10 liter/min, dry temperature 325 °C; skimmer 40 V, cap exit 50 V, Oct 1 DC 12 V, Oct RF 152.8 Vpp, Oct 2 DC 1.70 V, lens 1 -5.0 V, trap drive 44.7, lens 2 -60.0 V; target 30000, maximum acquisition accu time 200 ms, scan 100–550 *m/z*, average 5. MS detection was initiated at 4.0 minutes to allow the salts to be eluted to waste.

Microdialysis

CMA/20 microdialysis probes (CMA Microdialysis, Inc., North Chelmsford, MA) with a 10 mm, 100 kDa MWCO polyethersulfone (PES) membrane were used for all experiments. During the sampling process, the probes were perfused with PBS buffer using a CMA microdialysis pump (CMA/102) with 1-ml syringes (CMA Microdialysis, Inc.) at 1.0 μl/min. MCP-1 was prepared with PBS buffer containing 0.1% BSA.

In Vitro Delivery of Dexamethasone 21-Phosphate

The stability and extraction efficiency of dex-phos through the PES membranes was tested by immersing the PES microdialysis probes in a PBS solution held at 37 °C in a sand bath. dex-phos solutions (0.8 mg/ml, 0.5 mg/ml, and 0.2 mg/ml) in PBS were delivered at 1 μl/min through three different PES probes with randomized concentration order. This was performed to ensure there were not nonspecific adsorption problems with the use of dex-phos. Dialysates were collected from each probe every 30 minutes for a duration of 180 minutes (six samples). The concentrations of dex-phos in the perfusion fluid were measured before and after the infusion experiments. All samples were stored at 4 °C after sample collection and were analyzed within 24 hours.

In Vivo Experiments

In each experimental group, six male Sprague-Dawley rats (175–200 g; Taconic, NY) were used. The rats had free access to food and water and were subjected to a 12-hour on/off light cycle. All surgical protocols were approved by the Albany Medical College Institutional Animal Care and Use Committee and met the National Institutes of Health guidelines for care and use of experimental animals. Before implantation, the surgical tools were autoclaved and the microdialysis probes were

perfused with 70% ethanol followed by sterile water in a biosafety cabinet. Animals were anesthetized using isoflurane in a fume hood.

Under isoflurane anesthesia, aspectic technique, and temperature control (CMA/150, CMA Microdialysis, Inc.), two identical microdialysis probes were implanted into the subcutaneous space on each side of the dorsal spine. Probes were implanted by making a small incision with a scalpel followed by introduction of the microdialysis sampling probe into the subcutaneous space using the accompanying needle introducer provided by the manufacturer. Outlet tubing lines were tunneled under the skin towards the nape of the neck. A small incision allowed for exit of these tubing lines. The incisions were closed using surgical staples. After surgery, the animals were immediately placed and secured within the CMA/120 freely moving animal system (CMA Microdialysis, Inc.).

The probes were then flushed with the perfusion fluid at a rate of 5 μl/min for 5 minutes to ensure the fluid lines were full and cleared; the flow was then reduced to 1 μl/min. Following a 10-minute equilibration, dialysates were collected every 30 minutes for 3 hours.

Modulator Studies

Six rats were assigned to each group, i.e., dex-phos or MCP-1 delivery. In each group, probes were implanted in pairs where one probe served as a control and the other was perfused with the modulator. Each probe (control and modulator-containing) included the internal standards of VB_{12} (100 μM), 2-DG (5 mM), and antipyrine (100 μM). These concentrations were chosen to match the approximate concentration of glucose (2-DG) or for the ability to detect them using liquid chromatography-ultraviolet. In the modulator-probes, either MCP-1 (200 ng/ml) or dex-phos (0.2 mg/ml) was included in the perfusion fluid and infused locally. The MCP-1 concentration was chosen based on past cell culture studies that showed migration of monocytes to the probe containing the 200 ng/ml concentration.[39] The dexamethasone concentration was estimated on past reports that used controlled release along with the *EE* through the microdialysis probe.[40] Samples were collected every 30 minutes for 3 hours at a flow rate of 1 μl/min. The probes were only perfused during the sample collection periods. After sample collection, the tubing was carefully flushed with distilled water and the tubing lines were connected. These connected lines were then placed in a pouch created at the base of the neck of the animal and were connected via surgical staples that could be easily removed each experimental day with light anesthetic. All probes were sampled every other day until it was not possible to collect samples from the probes, e.g., probe failure. Probes were then explanted, taking care to remove a significant amount of the tissue surrounding the implant.

Histological Analysis

Probes exhibiting a visible capsule upon explantation were subjected to further histological analysis. Note that probes that were infused with dex-phos slid out of the capsular material upon explantation. Histological analysis of the explanted microdialysis probes with the surrounding tissue was performed using Masson's trichrome staining of paraffin-embedded sections.[41] Paraffin embedded tissue (with the microdialysis probe inside) was dehydrated (70, 85, 90, 100% ethanol over 2 hours) followed by formaldehyde fixation (overnight, 4 °C). Tissue samples were then infiltrated with a Citrosolv (Fisher Scientific, Franklin Lakes, NJ) and paraffin mixture (50:50, 1 hour) and embedded in 100% paraffin (3 × 1 hour) using an EG 1160 embedding apparatus (Leica). The embedded tissue was cut into 6-μm sections (Leica PM 2135; Leica) and the sections were floated in a flotation bath (Fisher Scientific), placed onto Superfrost slides (Fisher Scientific), and the sections dried onto the slides using a slide warmer (Fisher Scientific). For staining, slides were deparaffinized, rehydrated, and stained with Masson's trichrome according to the manufacturer's instructions (American Master Tech Scientific, Lodi, CA).

Statistical Analysis

All statistical analyses were performed using GraphPad Prism 4.00. The *EE*s for each internal standard and the glucose concentrations in the dialysates were grouped by their sampling date and then compared among the collection time points (six rats at each collection point on each sampling day for each analyte) using analysis of variance (ANOVA) for repeated measures. The ANOVA was applied among all the sampling days and between the control and modulator- perfused probes. When allowed by the ANOVA values and needed to determine the appropriate significance, a post-test (Tukey's test) to compare all pairs of sampling days was applied. The corresponding nonparametric repeated ANOVA, Friedman test, and Dunn's post-test were also performed on all the analytes to confirm the results by ANOVA for repeated measures, which relies on the assumption that the data has a normal distribution. Paired *t*-test was used to compare extraction efficiency values and concentrations between

the modulator vs control probes within the same sampling day.

Results

In Vitro Studies

The *EE* for MCP-1 delivery into the culture medium has been determined to be 13 ± 4% (n = 6 samples).[39] Based on this *in vitro* analysis, inclusion of 200 ng/ml MCP-1 in the dialysis perfusion fluid results in an approximate loss of approximately 20 ng/ml (20 pg/min at 1.0 µl/min) MCP-1 across the dialysis probe.

For different dex-phos concentrations, the *EE* values for delivery were 44 ± 5% (0.8 mg/ml standard), 42 ± 7% (0.5 mg/ml standard), and 47 ± 5% (0.2 mg/ml standard), (n = 18 from three probes), respectively. The repeated ANOVA measures showed no statistical difference among each collection time point among each of the concentrations. dex-phos (1 mg/ml) has been shown to be stable for 28 days at 4 and 22 °C.[42]

Detection of Dexamethasone from Collected In Vivo Dialysates

To confirm if dex-phos was being converted to dexamethasone, samples from the 3-day implanted microdialysis probes were injected directly into the HPLC-ESI/MS/MS system and quantified using appropriate standards. A chromatogram run using positive-ion mode on the ESI-MS of the *in vivo* dialysate shows the presence of antipyrine, dexamethasone, and dex-phos (**Figure 2**). The molecular identity of each peak was confirmed by retention time as well as mass spectrometric fragmentation patterns. Antipyrine, dexamethasone, and dex-phos were quantified from dialysate chromatograms from the dex-phos-releasing probe. This shows that dexamethasone was produced during the *in vivo* delivery of the dex-phos.

Internal Standard Analysis during Modulator Infusion

MCP-1

Table 2 lists the *EE* values obtained for the internal standards antipyrine, 2-DG, and VB_{12} from control and MCP-1-infused dialysis probes. For the control probes, VB_{12} *EE* was dramatically reduced from day 0 (28 ± 4%, n = 6 rats with 6 samples per rat) to day 12 (8 ± 4%, n = 3 rats with 6 samples per rat). Significant alterations in the *EE* were observed for the control probe at 5 days postimplantation vs 3 days for the MCP-1-infused probes when compared to the *EE* obtained on the implant day (day 0). No significant differences were observed for the *EE* values for antipyrine (63 ± 8%, n = 198 total samples, averaging all the data collected during the implantation period) among the different sampling days and 2-DG (55 ± 9%, n = 198 total samples) among the different sampling days. However, the collected glucose concentrations in the dialysates decreased significantly

Table 2.
MCP-1-Treated vs Control Delivery (*EE*%) of VB_{12}, Antipyrine, and 2-DG, and Glucose Concentration (mM)[a]

Day	VB_{12} (*EE*%)		Antipyrine (*EE*%)		2-DG (*EE*%)		Glucose (mM)	
	MCP-1	Control	MCP-1	Control	MCP-1	Control	MCP-1	Control
0	28 ± 3	28 ± 4	61 ± 6	60 ± 5	53 ± 5	52 ± 9	4.5 ± 0.8	4.5 ± 0.7
3	21 ± 5[b]	23 ± 3	64 ± 6	67 ± 7	58 ± 5	61 ± 5	4.3 ± 0.9	4.9 ± 0.8
5	17 ± 5[c]	20 ± 3[b]	63 ± 5	63 ± 6	57 ± 6	61 ± 4	3.4 ± 0.8	3.5 ± 0.5
7	11 ± 3[c]	16 ± 5[c]	61 ± 7	64 ± 5	50 ± 8	57 ± 2	2.1 ± 0.8[c,d]	3.2 ± 0.5
10	—	11 ± 3[c]	—	60 ± 7	—	45 ± 10	—	2.1 ± 1.0[c]
12 (n = 3)	—	8 ± 4	—	69 ± 6	—	44 ± 4	—	1.3 ± 0.6

[a] Two PES microdialysis probes were implanted into the dorsal subcutaneous space on day 0 (n = 6 rats). The control probe (Control) was perfused with 100 µM VB_{12}, 100 µM antipyrine, and 5 mM 2-DG on every other day at 1 µL/min for 3 hours to collect samples every 30 minutes while the animals allowed for moving freely. MCP-1 (200 ng/ml) was perfused with the same solution every other day through the other probe. The *EE*s reflect the delivery of analytes from the perfusates into the tissue space. Six points were collected to give a mean +/- standard deviation for each rat. These means were then averaged for all six rats. Data represent mean ± standard deviation, n = 6 rats. On day 12, only three control probes were still working properly.
[b,c] Data were significantly different from the data obtained on day 0 (acute day) at the 95% and 99% confidence levels, respectively, using only data with n = 6 rats, e.g., day 12 was not included in the ANOVA for repeated measures analysis.
[d] Denotes a difference between MCP-1 and control using a paired t-test at 95% confidence.

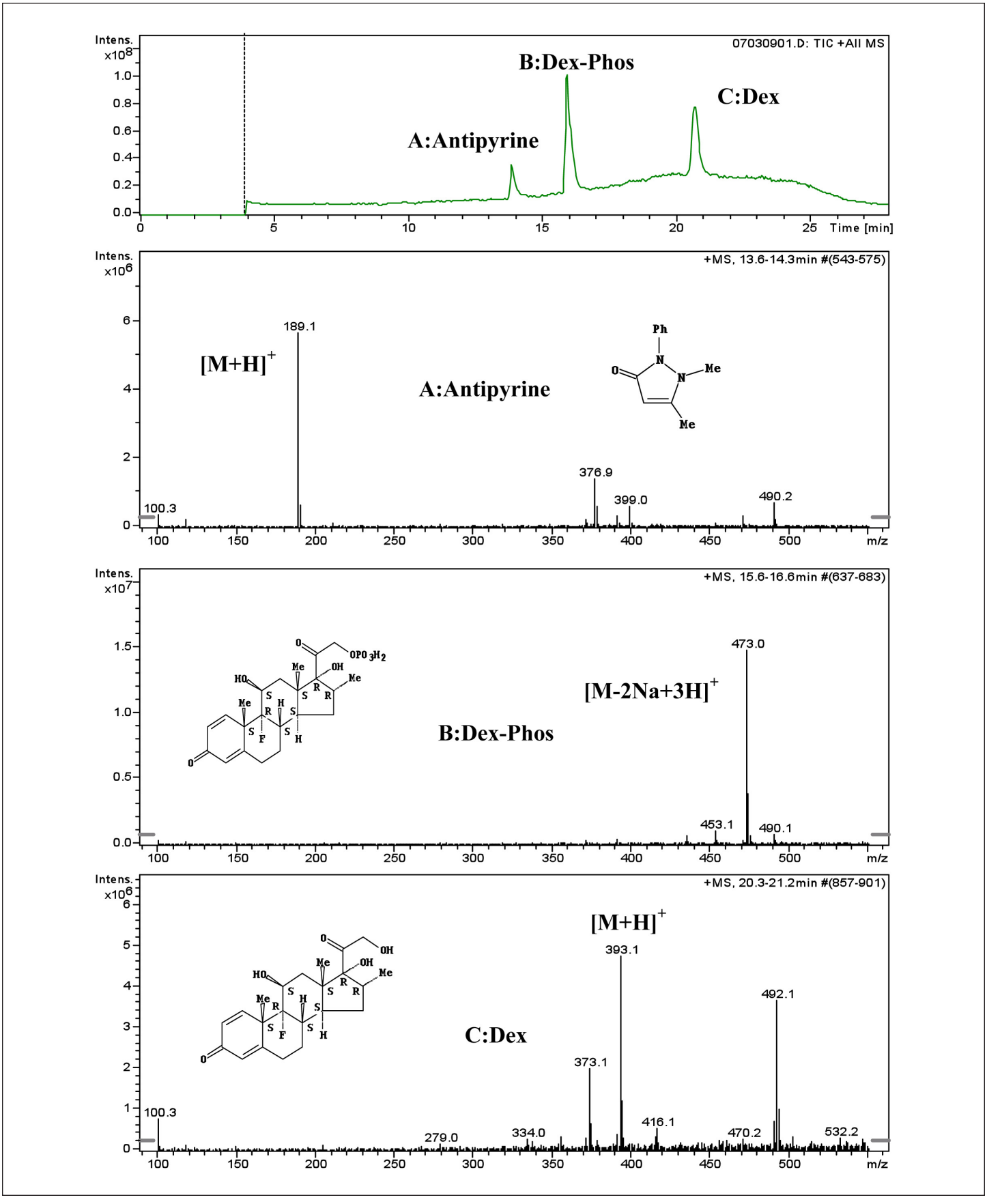

Figure 2. Positive ESI-MS spectra of antipyrine (peak A), dex-phos (peak B), and dexamethasone (dex) (peak C).

from day 0 (4.5 ± 0.7 mM, n = 36 samples from 6 rats) to implant day 12 (1.3 ± 0.6 mM, n = 18 samples from 3 rats), but were similar in terms of the overall reduction in collected concentrations between the two treatment groups. However, it is important to note that blood glucose values were not obtained in the awake and freely-moving animals. Only on day 7 did the glucose concentration collected from the MCP-1 containing probe differ from that obtained in the control probes.

Interestingly, all six microdialysis probes containing MCP-1 failed, e.g., no fluid could be passed through them, when sampled at the 10-day post implantation time point. This is in contrast to the control probes (n = 6 rats) that were functional 10 days postimplantation with three probes lasting to 12 days.

Dexamethasone

Conversion of dex-phos to dexamethasone. For dex-phos-infused probes, **Figure 3** shows the *EE%* for each sampling day as well as the concentration of collected dexamethasone detected in the microdialysis sampling probe. The *EE%* did not change during the sampling days. However, throughout the lifetime of the implants, the amount of dexamethasone recovered back into the microdialysis sampling probes increased steadily. The collected dexamethasone that was formed *in vivo* due to metabolism of dex-phos began to increase at day 5 postimplantation and reached a concentration of 0.118 ± 0.011 mg/liter (n = 6 rats), which was almost five times higher than the collected concentrations on the initial implantation day.

EE% and glucose concentrations. **Table 3** shows average *EE* of internal standards and the collection of glucose from control vs dex-phos-infused probes. For antipyrine and 2-DG infusions, there was no observed difference

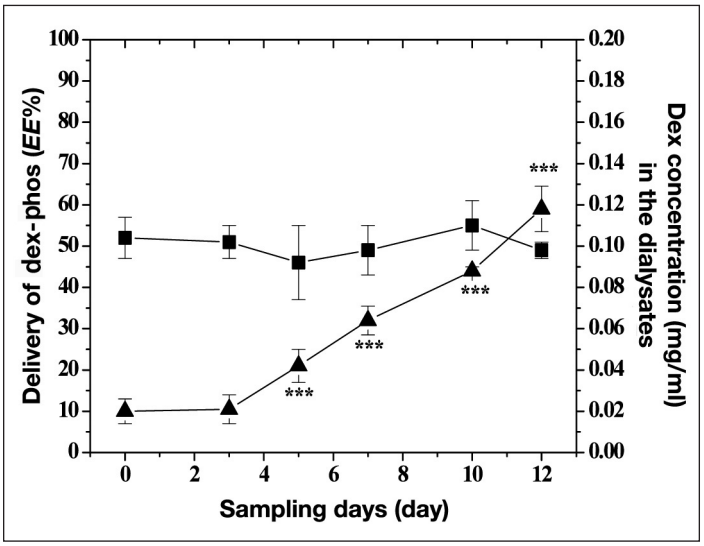

Figure 3. Delivery (EE%) of dex-phos (0.2 mg/ml) (■) and collected dex (mg/ml) in the dialysates (▲) at 1.0 μl/min through implanted microdialysis probes (PES) into the tissue space in freely moving rats (n = 6 animals). The *** labeled data were significantly different from data obtained on day 0 (acute day) at the 99.9% confidence level.

Table 3.
Delivery (*EE%*) for VB_{12}, Antipyrine, and 2-DG, and Glucose Concentration (mM) for Dialysates between Control and Dex-Phos-Releasing Probes[a]

Day	VB_{12} (*EE%*)		Antipyrine (*EE%*)		2-DG (*EE%*)		Glucose (mM)	
	Control	Dex-phos	Control	Dex-phos	Control	Dex-phos	Control	Dex-phos
0	29 ± 3	29 ± 4	63 ± 9	64 ± 5	65 ± 9	68 ± 8	5.0 ± 1.3	4.3 ± 0.8
3	25 ± 3	27 ± 3	59 ± 10	65 ± 5	64 ± 7	66 ± 5	4.5 ± 1.0	5.0 ± 0.4
5	19 ± 3[d,e]	24 ± 3[b]	61 ± 7	62 ± 4	62 ± 5	62 ± 6	4.0 ± 0.6	4.2 ± 0.6
7	14 ± 2[d,e]	21 ± 3[d]	58 ± 2	60 ± 11	56 ± 4	58 ± 6	3.4 ± 0.5[b]	3.7 ± 1.3
10	8 ± 1[d,e]	13 ± 3[d]	52 ± 7	55 ± 7	51 ± 5[b]	52 ± 7[c]	1.5 ± 0.3[d]	1.8 ± 0.4[d]
12	—	8 ± 1[d]	—	52 ± 12	—	38 ± 10[d]	—	0.8 ± 0.5[d]
14 (n = 2)	—	8	—	50	—	42	—	0.4

[a] Two PES microdialysis probes were implanted into the dorsal subcutaneous space on day 0 (n = 6 rats). One of the probes was perfused with 100 μM VB_{12}, 100 μM antipyrine, and 5 mM 2-DG as control. The other probe was perfused with the same internal standards and dex-phos (0.2 mg/ml). Both probes were sampled on every other day at 1 μl/min for 3 hours to collect samples every 30 minutes while the animals were allowed for moving freely. The *EE*s reflect the delivery of analytes from the perfusates into the tissue space and all the values were averaged over the average of the six collection points of all the 6 rats. Data represent mean ± standard deviation, n = 6 rats.
[b,c,d] Data were significantly different from the data obtained on day 0 (acute day) at 95%, 99%, and 99.9% confidence level, respectively.
[e] Denotes a difference between dex-phos and control using a paired t-test at 95% confidence.

in the *EE* values between the control and dex-phos-infused probes when comparing values obtained from each sampling day compared to the implantation day. However, by day 10 postimplantation, both the control and dex-phos-infused probes were exhibiting statistically significant differences in *EE* for the 2-DG infusion compared with the implantation day. For the control probe, this level was 51 ± 5% on day 10 vs 65 ± 9% on the implantation day ($p < .05$, $n = 6$ rats). For the dex-phos-infused probes, the decrease in 2-DG *EE%* was similar to that for controls at 10 days vs the implant day (68 ± 8% day 0 vs 52 ± 7% day 10, $p < .01$, $n = 6$ rats). By day 12, the control probes were no longer functioning. For the dex-phos-infused probes, the difference in the *EE* for 2-DG was now significant at the $p < .001$ level with an average value of 38 ± 10% ($n = 6$ rats). By day 14, only two probes that contained dex-phos were still functioning and the average *EE* for 2-DG was 42%.

The *EE* values and trends obtained for VB_{12} during the dex-phos infusions were similar to those obtained with the MCP-1 infusions. With the infusion of dex-phos, there was still a reduction in *EE* values throughout the implantation time. For both the control and dex-phos-infused probes, this reduction in *EE* occurred on day 5 postimplantation vs the implantation day. With the exception of day 5 postimplantation for the dex-phos-infused probe where $p < .05$, all other *EE* values obtained at later time points were statistically significant at the $p < .001$ levels. Additionally, by day 5 and beyond, VB_{12} *EE* was greater for dex-phos-releasing probes vs controls during each sampling day, $p < .05$. By day 7 and 10, there was a significant difference in the *EE%* of VB_{12} obtained from the dex-phos-releasing probes vs controls on each of these days.

Similar to the MCP-1 infusions, the glucose concentrations obtained in dialysates during dex-phos delivery were reduced with increasing implantation time but were not different when compared at each individual sampling day via a paired *t*-test. By day 10, the control and the dex-phos-infused probes had glucose concentrations that were well below the initial average concentrations of 5 mM obtained on the day of implantation.

Histological Analysis

MCP-1-Delivered Probe Histology

Figure 4.1 shows a photograph of the explanted probes (MCP-1-infused and control). MCP-1-infused probes have a much larger capsule than the controls. **Figures 4.2** and **4.3** show the respective Masson's trichrome staining on the tissue surrounding the explanted control (**Figure 4.2**) and MCP-1-delivered (**Figure 4.3**) probes using a 10× objective.

The control probe (**Figure 4.2**) has a compact cell layer surrounding the dialysis membrane (~150–200 μm, **Figure 4.2.2**, 20× objective) followed by a thin collagen layer (50 μm), and then an outer layer full of blood vessels (**Figure 4.2.1**, 20× objective). For the MCP-1-delivered probe, a more extensive cell layer was observed adjacent to the dialysis membrane (~150–200 μm, **Figure 4.3.1**, 20× objective). Together with the larger cellular layer, a rich collagen layer (**Figure 4.3.2**, 20× objective) embedded with blood vessels (**Figure 4.3.3**, 20× objective) composed the capsule (500–600 μm) that surrounded the probes that delivered MCP-1.

Dexamethasone

The microdialysis probes (control and dex-phos-infused) were explanted 14 days after implantation. However, the probes that were infused with dex-phos were difficult to handle because they were more fragile and tended to fragment during the sectioning processes needed for histological preparation for Masson's trichrome staining. Generally, poor histological images were obtained for these samples. The fragile nature of these capsules was due to reduced fibrosis that was visible upon inspection after probe explantation.

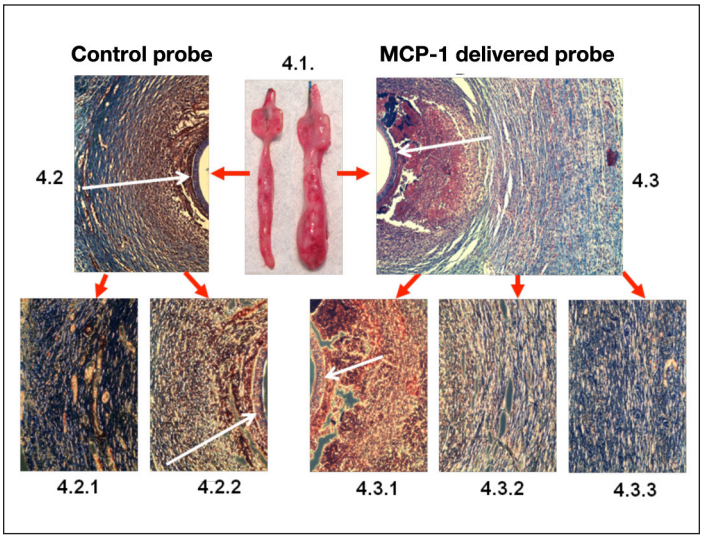

Figure 4. Masson's trichrome staining on the tissue surrounding the 14-day implanted microdialysis probe (PES) membranes in the dorsal subcutaneous space. The white arrows are pointing to the probe membrane. The probe on the left in **Figure 4.1** was the control probe **(Figure 4.2, 4.4.1, 4.2.2)** and the probe on the right was the MCP-1-delivered probe **(Figure 4.3, 4.3.1, 4.3.2, 4.3.3). Figures 4.2** and **4.3** were observed using a 10× objective. A 20× objective was used to obtain **Figures 4.2.1, 4.2.2, 4.3.1, 4.3.2, and 4.3.3.**

Discussion

Addressing the biological barriers to long-term implantable sensing has been of significant interest for decades and has been related principally to glucose sensing.[43] Additionally, there has been research related to improving drug delivery devices by reducing the thickness of the capsule created during the foreign body reaction.[30] The work described here modified the size of the capsule surrounding the microdialysis sampling probe and assessed the effect of that size on mass transport properties into and out of the microdialysis sampling device.

This study focused on understanding how different factors during the foreign body response may interfere with the performance of an *in vivo* sensor/device. It is known that a biological barrier exists with respect to solute mass transport.[44–47] However, it is not known how solute mass transport is influenced by the different biological modulation approaches.

MCP-1 is a known chemoattractant and thus was used to recruit inflammatory cells and generate a large foreign body response. In this work, the rationale for using MCP-1 was as a positive control to attract monocytes rather than to determine an exact dosage or change in dosage over time. Indeed, the capsule surrounding MCP-1-infused probes was nearly twice the thickness (500–600 μm) as that of control probes (200–225 μm) (**Figure 4**). Mononuclear cells were observed as shown in **Figures 4.3.1** and **4.3.2**, and these cells occupied more than one third of the entire capsule surrounding the MCP-1 delivered probes. In other work, it has been shown that an extensive amount of granulation tissue that accumulates during inflammation induced by lipopolysaccharide can be attributed to MCP-1.[48] Studies with implanted hollow fibers, some with similar chemistry to microdialysis membranes, have noted extensive material degradation after implantation.[49]

Despite being encapsulated by a larger capsule than control or dex-phos-releasing probes, the probes containing MCP-1 did not exhibit significant differences in their *EE%* for the delivered internal standards, antipyrine, VB_{12}, or 2-DG. Furthermore, the collected glucose concentration during each sampling day from MCP-1-containing probes was not different compared to the control probes with the exception of day 7 (**Table 2**). While Norton and colleagues observed an approximate 20% reduction in glucose concentrations at 8 days postimplantation for bare PES-membrane microdialysis probes as well as hydrogel-modified probes, they did not report glucose recovery with their dexamethasone- or vascular endothelial growth factor (VEGF)-releasing materials.[24] We observed an approximate 70% reduction in collected glucose concentrations by day 7.

Further work is necessary to verify how the distance of capillaries from the probe affects glucose supply during microdialysis sampling collection. The microdialysis sampling of glucose from human adipose tissue during long-term implantation from another paper came to the same conclusion.[44,50] Thus collection of glucose at the implant site may depend on how close the capillaries are to the implanted microdialysis probes. Novak and colleagues modeled the sensitivity of different parameters and found that capsule thickness strongly influenced time lag and capillary density, and overall porosity of the capsule (volume fraction) affects glucose concentrations at the sensor site.[10] In other words, supply of glucose is critical.

The probes that delivered dex-phos had tissue structures that were quite fragile with respect to explanation causing difficulties with obtaining significant histological analysis. However, these probes did not exhibit any differences in the *EE%* for the internal standards when compared to controls. Additionally, there were no improvements with the dex-phos-releasing probes in terms of glucose concentrations collected as compared to controls. However, the *EE%* of VB_{12} was improved at day 5 compared to controls in this group and the probes remained viable in terms of their ability to collect or deliver solutes for 2 days longer than controls. This extension in viability of the probe for delivery of the larger molecular weight VB_{12} may be due to the fragile and smaller capsules produced around the dialysis probe in the presence of dexamethasone. The antipyrine, glucose, and 2-DG data show that despite large capsules, these smaller molecular weight compounds are still capable of passing through the capsule. However, a combination of both size and possibly ionic interactions of VB_{12} with capsular tissue may affect its transport in controls as compared to dex-phos-infused probes.

Additionally, the increased amount of dexamethasone recovered back into the dialysis probe as a function of time may be due to increased esterase activity that is caused during a foreign body reaction that has been reported by others.[51] The overall increased proteolytic activity caused during the inflammatory response may affect the dialysis probes by degrading or destroying different probe components, especially the tubing connections that are glued. The reduction in the inflam-

matory response and fibrosis may have led to the increased lifetime of the implanted microdialysis sampling probe.

Other studies have demonstrated that the monocyte infiltration at the implant site is reduced by releasing dexamethasone,[52,53] but no evidence was provided regarding the variation of capsule thickness or the formation of collagen or blood capillaries. With the exception of day 7 and 10 VB_{12} *EE%*, the calibration of implanted microdialysis probes was not altered as compared to controls when locally infusing dex-phos.

The lack of change in *EE%* for the dex-phos-infused probes vs controls may be due to the reported inhibition of angiogenesis by corticosteroids.[54] Dexamethasone has been shown to reduce VEGF expression.[55,56] VEGF is believed to regulate the induction of angiogenesis. The lack of blood supply to the dialysis probe would be expected to affect the *EE%* values for substances as they may not be cleared as rapidly from the space. In microdialysis terms, this would suggest that diffusion through the capsule is not especially limiting and that blood flow surrounding the probe is one of the most significant factors for the extraction efficiency of the internal standards used in this study. This lack of blood supply may also be a reason for the higher concentrations of dexamethasone observed in **Figure 3** since it may not be removed at later time points.

While there are reported limitations with the rat model as compared to humans due to observed differences in the types of tissue density between lean subcutaneous tissue in a rat and humans that have adipose tissue,[23,57] how the foreign body reaction affects transport can be observed in the rat model. From the glucose collections, the supply of glucose to the dialysis probe seems to be affected at longer time periods, as would be expected. The differences in *EE%* observed between the smaller molecular standards, antipyrine, 2-DG, and VB_{12}, suggest interesting mass transport properties exist within the capsule. For long-term implants, antipyrine also exhibits supply reduction when given externally rather than delivered locally through the microdialysis probe.[27] Additionally, it should be noted that all three of the internal standards could also be used for human studies with microdialysis sampling.

Finally, the necessary supply of glucose with this model may be exaggerated due to the flux differences between the removal of material by a dialysis probe vs what would be expected from a typical glucose sensor. A typical amperometric glucose sensor consumes an estimated 30 pmol/s of glucose when immersed in 5 mM glucose solutions.[6] The flux through a microdialysis probe is significantly higher than this value. With an estimtated 60% *EE* for glucose (based on 2-DG's values), a microdialysis probe immersed in a 5 mM solution with a 1.0 μl/min flow rate would remove 50 nmol/s of glucose. The biological system then must keep up with this greater than 1000-fold difference in removal due to the flux differences for glucose to maintain steady-state concentrations that are observed in the dialysis data.

Conclusions

In these studies, microdialysis sampling probes served the dual purpose of releasing modulating agents and recovering solutes from a localized implantation site. This strategy allowed us to test for alterations in the *in vivo* calibration of the microdialysis sampling probes. No differences in localized delivery (release) of antipyrine, 2-deoxyglucose, or VB_{12}, or recovery of glucose were observed between control and MCP-1-containing probes despite an increase in the thickness of the capsule produced in the presence of MCP-1. Similarly, dex-phos-releasing probes did not have a reduction in their capsule thickness as compared to controls but had capsules that appeared to be less fibrotic because of the fragile nature of their capsule. The small changes in *EE%* found for conditions with wide variations in capsule thickness suggest that the cell layer close to the probe is supplied by capillaries that maintain probe function. Microdialysis sampling methods have been successfully employed to study how modulation of the foreign body response affects *in vivo* extraction efficiency.

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References:

1. Wilson GS, Johnson MA. *In-vivo* electrochemistry: what can we learn about living systems? Chem Rev. 2008;108(7):2462-81.
2. Garris PA. Advancing neurochemical monitoring. Nat Methods. 2010;7(2):106-8.
3. Fraser DM, editor. Biosensors in the body, Continuous *in vivo* monitoring. Chichester (UK): John Wiley & Sons; 1997.
4. Reach G, Choleau C. Continuous glucose monitoring: physiological and technological challenges. Curr Diabetes Rev. 2008;4(3):175-80.
5. Brauker J. Continuous glucose sensing: future technology developments. Diabetes Technol Ther. 2009;11(Suppl 1):S25-36.
6. Cunningham DD, Stenken JA, editors. *In vivo* glucose sensing. Hoboken (NJ): John Wiley & Sons, Inc.; 2010.
7. Wilson GS, Gifford R. Biosensors for real-time *in vivo* measurements. Biosens Bioelectron. 2005;20(12):2388-403.
8. Anderson JM, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. Semin Immunol. 2008;20(2):86-100.

9. Wisniewski N, Moussy F, Reichert WM. Characterization of implantable biosensor membrane biofouling. Fresenius J Anal Chem. 2000;366(6-7):611-21.

10. Novak MT, Yuan F, Reichert WM. Modeling the relative impact of capsular tissue effects on implanted glucose sensor time lag and signal attenuation. Anal Bioanal Chem. 2010;398(4):1695-705.

11. Hoss U, Jeddi I, Schulz M, Budiman E, Bhogal C, McGarraugh G. Continuous glucose monitoring in subcutaneous tissue using factory-calibrated sensors: a pilot study. Diabetes Technol Ther. 2010;12(8):591-7.

12. Gant RM, Hou Y, Grunlan MA, Cote GL. Development of a self-cleaning sensor membrane for implantable biosensors. J Biomed Mater Res A. 2009;90(3):695-701.

13. Ward WK. A review of the foreign-body response to subcutaneously-implanted devices: The role of macrophages and cytokines in biofouling and fibrosis. J Diabetes Sci Technol. 2008;2(5):768-77.

14. Yu B, Ju Y, West L, Moussy Y, Moussy F. An investigation of long-term performance of minimally invasive glucose biosensors. Diabetes Technol Ther. 2007;9(3):265-75.

15. Hetrick EM, Schoenfisch MH. Reducing implant-related infections: active release strategies. Chem Soc Rev. 2006;35(9):780-9.

16. Nandi P, Lunte SM. Recent trends in microdialysis sampling integrated with conventional and microanalytical systems for monitoring biological events: a review. Anal Chim Acta. 2009;651(1):1-14.

17. Westerink BHC, Cremers TIFH, editors. Handbook of microdialysis sampling: methods, applications, and clinical aspects. Amsterdam: Academic Press; 2007.

18. Bungay PM, Morrison PF, Dedrick RL. Steady-state theory for quantitative microdialysis of solutes and water *in vivo* and *in vitro*. Life Sci. 1990;46(2):105-19.

19. Bungay PM, Newton-Vinson P, Isele W, Garris PA, Justice JB. Microdialysis of dopamine interpreted with quantitative model incorporating probe implantation trauma. J Neurochem. 2003;86(4):932-46.

20. Stenken JA. Methods and issues in microdialysis calibration. Anal Chim Acta. 1999;379:337-58.

21. Henninger N, Woderer S, Kloetzer H-M, Staib A, Gillen R, Li L, Yu X, Gretz N, Kraenzlin B, Pill J. Tissue response to subcutaneous implantation of glucose-oxidase-based glucose sensors in rats. Biosens Bioelectron. 2007;23(1):26-34.

22. Ju YM, Yu B, West L, Moussy Y, Moussy F. A novel porous collagen scaffold around an implantable biosensor for improving biocompatibility. II. Long-term *in vitro/in vivo* sensitivity characteristics of sensors with NDGA- or GA-crosslinked collagen scaffolds. J Biomed Mater Res A. 2010;92(2):650-8.

23. Wisniewski N, Rajamand N, Adamsson U, Lins PE, Reichert WM, Klitzman B, Ungerstedt U. Analyte flux through chronically implanted subcutaneous polyamide membranes differs in humans and rats. Am J Physiol. 2002;282(6):E1316-23.

24. Norton LW, Koschwanez HE, Wisniewski NA, Klitzman B, Reichert WM. Vascular endothelial growth factor and dexamethasone release from nonfouling sensor coatings affect the foreign body response. J Biomed Mater Res A. 2007;81(4):858-69.

25. Bridges AW, Whitmire RE, Singh N, Templeman KL, Babensee JE, Lyon LA, Garcia AJ. Chronic inflammatory responses to microgel-based implant coatings. J Biomed Mater Res A. 2010;94(1):252-8.

26. Rodriguez A, Meyerson H, Anderson JM. Quantitative *in vivo* cytokine analysis at synthetic biomaterial implant sites. J Biomed Mater Res A. 2009;89(1):152-9.

27. Mou X, Lennartz MR, Loegering DJ, Stenken JA. Long-term calibration considerations during subcutaneous microdialysis sampling in mobile rats. Biomaterials. 2010;31(16):4530-9.

28. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res. 2009;29(6):313-26.

29. Derendorf H, Rohdewald P, Hochhaus G, Möllmann H. HPLC determination of glucocorticoid alcohols, their phosphates and hydrocortisone in aqueous solutions and biological fluids. J Pharm Biomed Anal. 1986;4(2):197-206.

30. Morais JM, Papadimitrakopoulos F, Burgess DJ. Biomaterials/tissue interactions: possible solutions to overcome foreign body response. AAPS J. 2010;12(2):188-96.

31. Ward WK, Hansen JC, Massoud RG, Engle JM, Takeno MM, Hauch KD. Controlled release of dexamethasone from subcutaneously-implanted biosensors in pigs: localized anti-inflammatory benefit without systemic effects. J Biomed Mater Res A. 2010;94(1):280-7.

32. Hazel JR, Sidell BD. A method for the determination of diffusion coefficients for small molecules in aqueous solution. Anal Biochem. 1987;166(2):335-41.

33. Snyder KL, Nathan CE, Yee A, Stenken JA. Diffusion and calibration properties of microdialysis sampling membranes in biological media. Analyst. 2001;126(8):1261-8.

34. Moussy Y, Dungel P, Hersh L. Diffusion of [3H]dexamethasone in rat subcutaneous slices after injection measured by digital autoradiography. Biotechnol Prog. 2006;22(6):1715-9.

35. Renkin EM. Filtration, diffusion, and molecular sieving through porous cellulose membranes. J Gen Physiol. 1954;38(2):225-43.

36. Altieri AS, Hinton DP, Byrd RA. Association of biomolecular systems via pulsed field gradient NMR self-diffusion measurements. J Am Chem Soc. 1995;117(28):7566-7.

37. Paavola CD, Hemmerich S, Grunberger D, Polsky I, Bloom A, Freedman R, Mulkins M, Bhakta S, McCarley D, Wiesent L, Wong B, Jarnagin K, Handel TM. Monomeric monocyte chemoattractant protein-1 (MCP-1) binds and activates the MCP-1 receptor CCR2B. J Biol Chem. 1998;273(50):33157-65.

38. Kanamori T, Sakai K, Awaka T, Fukuda M. An improvement on the method of determining the solute permeability of hollow-fiber dialysis membranes photometrically using optical fibers and comparison of the method with ordinary techniques. J Membr Sci. 1994;88(2-3):159-65.

39. Mou X. Modulation of foreign body response towards implanted microdialysis probes [Ph.D. dissertation]. Troy (NY): Rensselaer Polytechnic Institute; 2007.

40. Hickey T, Kreutzer D, Burgess DJ, Moussy F. Dexamethasone/PLGA microspheres for continuous delivery of an anti-inflammatory drug for implantable medical devices. Biomaterials. 2002;23(7):1649-56.

41. Bancroft J, Gamble M. Theory and practice of histological techniques. London (England): Churchill Livingstone; 2001.

42. Lugo RA, Nahata MC. Stability of diluted dexamethasone sodium phosphate injection at two temperatures. Ann Pharmacother. 1994;28(9):1018-9.

43. Woodward SC. How fibroblasts and giant cells encapsulate implants: considerations in design of glucose sensors. Diabetes Care. 1982;5(3):278-81.

44. Schoonen AJ, Wientjes KJ. A model for transport of glucose in adipose tissue to a microdialysis probe. Diabetes Technol Ther. 2003;5(4):589-98.

45. Sharkawy AA, Klitzman B, Truskey GA, Reichert WM. Engineering the tissue which encapsulates subcutaneous implants. III. Effective tissue response times. J Biomed Mater Res. 1998;40(4):598-605.

46. Thennadil SN, Rennert JL, Wenzel BJ, Hazen KH, Ruchti TL, Block MB. Comparison of glucose concentration in interstitial fluid, and capillary and venous blood during rapid changes in blood glucose levels. Diabetes Technol Ther. 2001;3(3):357-65.

47. Wisniewski N, Klitzman B, Miller B, Reichert WM. Decreased analyte transport through implanted membranes: differentiation of biofouling from tissue effects. J Biomed Mater Res. 2001;57(4):513-21.

48. Conti P, Feliciani C, Barbacane RC, Frydas S, Placido FC, Cataldo I, Reale M. Monocyte chemotactic protein-1 gene expression and translation in formed granulomatous calcified tissue *in vivo*. Calcif Tissue Int. 1999;64(1):57-62.

49. Clark H, Barbari TA, Stump K, Rao G. Histologic evaluation of the inflammatory response around implanted hollow fiber membranes. J Biomed Mater Res. 2000;52(1):183-192.

50. Wientjes KJ, Vonk P, Vonk-van Klei Y, Schoonen AJ, Kossen NW. Microdialysis of glucose in subcutaneous adipose tissue up to 3 weeks in healthy volunteers. Diabetes Care. 1998;21(9):1481-8.

51. Labow RS, Sa D, Matheson LA, Santerre JP. Polycarbonate-urethane hard segment type influences esterase substrate specificity for human-macrophage-mediated biodegradation. J Biomater Sci Polym Ed. 2005;16(9):1167-77.

52. Ito T, Fraser IP, Yeo Y, Highley CB, Bellas E, Kohane DS. Anti-inflammatory function of an in situ cross-linkable conjugate hydrogel of hyaluronic acid and dexamethasone. Biomaterials. 2007;28(10):1778-86.

53. Thieme TR, Mirkovich A, Maloney P, Goodwin DA. *In vivo* and *in vitro* effects of dexamethasone on leukocyte migration in the rat adjuvant arthritis model. Inflammation. 1982;6(4):371-86.

54. Auerbach W, Auerbach R. Angiogenesis inhibition: a review. Pharmacol Ther. 1994;63(3):265-311.

55. Nauck M, Karakiulakis G, Perruchoud AP, Papakonstantinou E, Roth M. Corticosteroids inhibit the expression of the vascular endothelial growth factor gene in human vascular smooth muscle cells. Eur J Pharmacol. 1998;341(2-3):309-15.

56. Edelman JL, Lutz D, Castro MR. Corticosteroids inhibit VEGF-induced vascular leakage in a rabbit model of blood-retinal and blood-aqueous barrier breakdown. Exp Eye Res. 2005;80(2):249-58.

57. Rajamand Ekberg N, Brismar K, Malmstedt J, Hedblad M-A, Adamson U, Ungerstedt U, Wisniewski N. Analyte flux at a biomaterial-tissue interface over time: implications for sensors for type 1 and 2 diabetes mellitus. J Diabetes Sci Technol. 2010;4(5):1063-72.