Macrophage and Encapsulation Effects to Glucose Recovery through Implanted Microdialysis Probes

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Statement of Purpose

The foreign body response is among the primary causes for glucose sensor loss of function during long term implantation. The purpose of this study is to use microdialysis sampling probes to understand how macrophages and implant encapsulation affect localized glucose concentrations surrounding an implanted device. Bi-directional analyte extraction efficiency (*EE*) across the microdialysis membrane is defined in equation 1, where 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

$$EE = \frac{C_{inlet} - C_{outlet}}{C_{inlet} - C_{sample}} \times 100 \qquad \textbf{Eq. 1}$$

EE is sensitive to tissue diffusive and kinetic parameters. An internal standard, 2-deoxyglucose (2-DG), is delivered through the dialysis probe to determine glucose uptake variation at the implant site. Antipyrine is used to determine capillary permeability alterations at the implant site.

Materials and Methods

Polycarbonate (PC, 10 mm with 20 kDa MWCO) or polyethersulfone (PES, 10 mm with 100 kDa MWCO) microdialysis probes were implanted into the dorsal subcutis (2 per animal) of male Sprague-Dawley rats (200 to 300 grams) for either three days (day 3) or acutely (day 0). Antipyrine (100 µM) and 2-DG (5 mM) were infused through the microdialysis probes. Samples were collected every 30 minutes for 3 hours. Glucose and 2-DG were quantified from diluted microdialysates (500 fold) using anion-exchange chromatography coupled with pulsed amperometric detection (IC-PAD). Antipyrine dialysate samples were analyzed by LC-UV without further pretreatment or dilution.

Results and Discussion

Two PC probes were implanted into the same rat on the same day of sample collection. One probe was infused with either 2-DG or antipyrine in a phosphate buffered saline solution (PBS), while the other probe was infused with PBS as a control. The 2-DG loss (*EE*%) was 45 ± 4% (n=6) and for antipyrine is 47 ± 2% (n=6). The glucose concentration from the control probe was 4.6 ± 0.2 mM (n=6).

For the three day implantation experiment, one PC probe was implanted three days before sample collection while another PC probe was implanted into the opposite side of the dorsal subcutis on the day of sample collection. Table 1 shows the loss of 2-DG and antipyrine for several different experiments. For rats A and B, both antipyrine and 2-DG was infused through the probes. For rats C and D the perfusate contained either antipyrine or 2-DG.

Table 1. Internal Standard Loss through Implanted PC Probes

		Antipyrine EE%	2-DG EE%	Glucose (mM)
Rat A	Day 0	53±1(n=5)*	46±4(n=5)	5.2±0.8(n=5)
	Day 3	62±3(n=5)*	48±9(n=5)	6.4±1.4(n=5)
Rat B	Day 0	37±3(n=6)*	32±7(n=6)	4.7±0.7(n=6)
	Day 3	57±2(n=6)*	39±9(n=6)	4.2±0.5(n=6)
Rat C&D	Day 0	52±3(n=6)*	38±7(n=5)	4.6±0.3(n=5)
	Day 3	60±2(n=6)*	35±2(n=5)	5.0±0.7(n=5)

* Between Day 0 and Day 3 $p<0.0001$

The antipyrine loss (*EE*) through PES probes implanted into the dorsal subcutis was 63 ± 1% and 55 ± 2% ($p=2\times10^{-4}$, n=6) for "day3" and "day 0", respectively. The concentration of glucose in the dialysates from "day 3" and "day 0" implanted PES probes were 8.4 ± 1.4 mM and 5.5 ± 0.4 mM (n=6).

Conclusions

The microdialysis sampling probes allow direct biochemical collection at the implant site. Simultaneous delivery of 2-DG and antipyrine through the dialysis probes may allow the relationship between potential cellular glucose uptake variation and the capillary permeation at the implant site to be elucidated. There are three potential explanations for the increase in the antipyrine microdialysis *EE* (loss) between the acutely implanted probes compared to the three-day implanted probes. First, a greater density of capillaries due to angiogenesis may surround the probe. Second, the capillaries may be more permeable around the probe. Third, the tissue may be initially damaged during the acute implant and may have settled and sealed around the probe after three days. Histological analyses as well as collection of growth factors (e.g., VEGF) will ultimately help reveal which explanation best fits this data.

It is interesting to note that no changes in 2-DG loss or glucose concentrations were observed between the two probes suggesting that tissue parameters that affect 2-DG loss from the probe were not altered. Longer term implantations (5 days, 7 days and 10 days) are being performed to further verify these findings.

Acknowledgements

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