

# The Linear Probe: A Flexible Choice for In Vivo Microdialysis Sampling in Soft Tissues

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*A new design of linear microdialysis probe was implanted in the thigh muscle of rat for 4 to 6 days. In vivo drug delivery and pharmacokinetics experiments were performed using this probe with acetaminophen as the test compound. The results demonstrated the durability of the new probe design and showed the utility of this probe for pharmacokinetic studies in awake, freely moving animals.*

For a decade, the use of microdialysis sampling in pharmacokinetics research has proven to have many benefits such as clean samples, more frequent samples, conservation of body fluid, and fewer animals per study. In addition, it provides a direct profile of pharmacokinetics within the tissue of interest instead of traditional methods which calculate the tissue concentration indirectly from serial blood samples.

Microdialysis sampling was originally developed by neuroscientists for CNS studies, but during the past several years its suitability for sampling from other sites has been demonstrated. The applications of microdialysis sampling in the neurosciences have been extensively reviewed (1,2).

Various designs of microdialysis probes have been developed

for particular sites or types of tissue. For example, the rigid cannula probe, most suitable for sampling from the brain, has also been used to sample from adipose tissue (3), muscle (3-5), and liver (3,6). A flexible cannula probe has proven most appropriate for sampling from blood (7,8), but has also been used in liver (9). A flow-through or shunt probe design has been used in the bile duct (9-11). As previously reported, linear design probes have been used for pharmacokinetics and metabolism studies of dermal tissue (12), muscle and tumor (13), and liver (14). The linear design has the advantages of minimizing tissue damage, being totally flexible (and therefore more comfortable), and sufficiently durable for use in awake, freely moving animals.

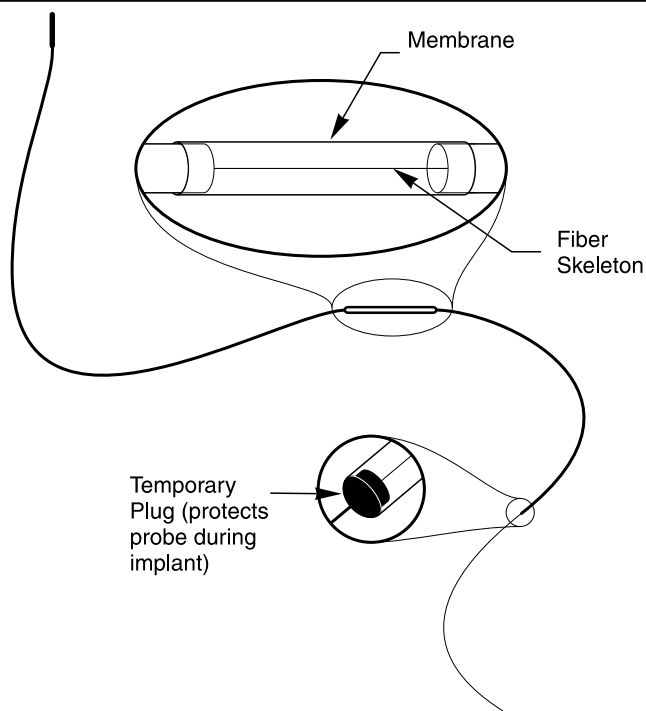
Recently, we developed a new linear microdialysis probe (**F1**)

with an even smaller profile and additional strength and flexibility. It samples the interstitial fluid of a variety of soft tissues including dermis, muscle, adipose and subcutaneous tissue, liver and tumors. Long lengths of highly flexible tubing in the probe make it possible to implant in tissues that are distant from the exit site for the inlet and outlet tubing. This feature also makes the probe inherently more comfortable for the animal than a probe that must be anchored to skin proximal to the implant site. This linear probe moves with the tissue and does not jab or tear tissue during normal respiration, digestion, or movement by the animal.

Implanting a probe in the thigh muscle of an active free-moving rat is a good test of ruggedness and stability. Linear probes in this muscle remained functional for up to 6

**F1**

*Linear Microdialysis Probe. The cross-sectional profile of this design is very small (180  $\mu\text{m}$ ) so that tissue damage is minimal. An internal, flexible fiber acts as a structural support for the probe. The fiber is also used to pull the probe into the sample tissue without straining the fragile membrane. Once the probe is implanted, the user has the option of removing the fiber. This decreases flow inside the probe at the same syringe pump setting and increases % relative recovery. The total length of the probe is 51 cm.*



days. Consistent probe behavior was determined by daily *in vivo* delivery of acetaminophen via the probe. Pharmacokinetics experiments were also performed on days 1 and 5 of the studies. Acetaminophen was chosen as a model analyte because its pharmacokinetics have been extensively studied (6,15-17).

### Experimental procedures

#### Chemicals

Acetaminophen was purchased from Sigma. HPLC grade acetonitrile was obtained from Fisher Scientific. All standards and solutions were prepared using purified water obtained from a NANOpure system. All other chemicals were reagent grade or better and were used as received.

#### Probes

LM-10 Linear Microdialysis Probes with a 10 mm membrane window were obtained from BAS (P/N MD-2000).

#### Liquid chromatography

A BAS 200 liquid chromatograph with internal UV detector setting of 250 nm and ChromGraph<sup>®</sup> data acquisition/analysis software were used. The Chrom-

Graph PKA Pharmacokinetics accessory was also used. Separation was achieved on a SepStik UniJet<sup>®</sup> (P/N MF-8949, BAS) ODS 3  $\mu\text{m}$  microbore column (1 mm x 100 mm). The mobile phase was 0.05 M ammonium phosphate buffer, pH 2.5, containing 8% acetonitrile by volume, at a flow rate of 60  $\mu\text{L}/\text{min}$ .

#### Sampling

The probe was continuously perfused with Ringer's solution or Ringer's/acetaminophen solution at a flow rate of 2.0  $\mu\text{L}/\text{min}$ . Microdialysate samples were injected directly into the LC system using an on-line injector with a 5  $\mu\text{L}$  loop at 10 min. intervals.

#### Surgical procedures

Male Sprague-Dawley rats weighing 450-480 g were anesthetized intramuscularly using ketamine and xylazine (80 mg/kg and 10 mg/kg, respectively). An incision was made in the skin to expose the thigh muscle. A linear probe was implanted in the muscle tissue of the thigh by inserting a 25-gauge needle through the muscle and inserting one end of the probe inlet tubing through the needle.

The needle was then withdrawn and the probe pulled through the tissue, placing the dialysis membrane fully inside the muscle. PE-50 tubing was cannulated into the femoral vein of the other leg for administration of the drug. The probe tubing and the cannula were tunneled under the skin and externalized at the center of the back of the neck. Following surgical procedures, the rat was maintained in an awake animal system, which allowed movement without tangling of fluid lines. The rat had free access to food and water throughout the experiment.

### In vitro probe calibration procedure

Recovery was determined in Ringer's solution spiked with a known concentration of acetaminophen. Delivery was determined by perfusing the probe with a solution of known concentration of acetaminophen and monitoring decrease in concentration in the probe effluent. Recovery and delivery *in vitro* were conducted in stirred solutions maintained at 37°C. At the beginning of each experiment, the probe was perfused for 30 min. prior to collecting five dialysate samples at 10 min. intervals. *In vitro* recovery and delivery were calculated as follows:

$$\text{Recovery \%} = C_d / C_i \times 100\%$$

$$\text{Delivery \%} = 1 - C_d / C_i \times 100\%$$

where  $C_d$  is the concentration of a given compound in dialysate and  $C_i$  is the concentration of the same compound in the initial standard solution. Since recovery and delivery are derived values, the standard deviations were calculated by propagation of errors.

### In vivo probe calibration procedure

*In vivo* delivery was performed in the same manner as the *in vitro* experiment except that the probe was implanted in the muscle. The initial delivery experiment began 3 to 4 hours after surgery (day 0) and was repeated daily.

**T1**

Calibration of  
Microdialysis  
Probe\*.

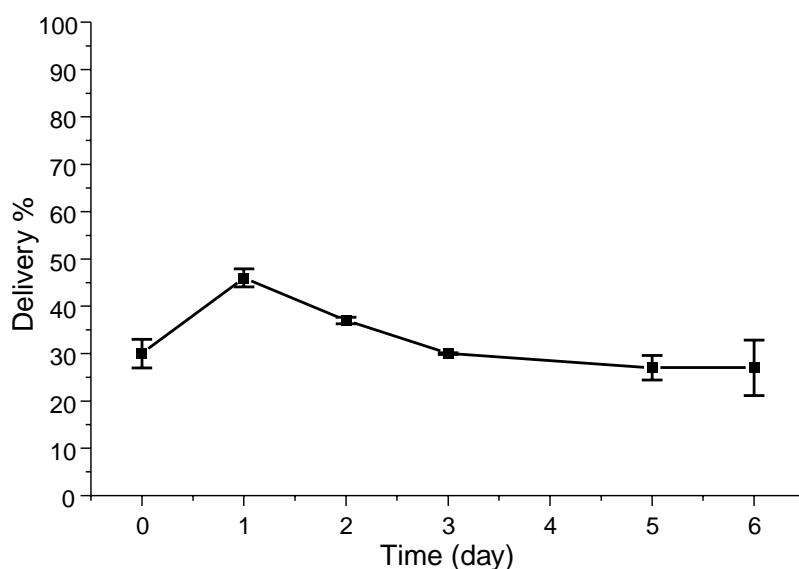
Probe No.	In Vitro Calibration		In Vivo Calibration (Delivery)					
	Recovery (%)	Delivery (%)	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
1	33.9 ± 0.1		48 ± 1	34 ± 1	24.7 ± 0.5	26 ± 4		
2	43 ± 2	47.7 ± 0.2**	43.6 ± 0.3	31 ± 1			29 ± 2	31.7 ± 0.5
3	63 ± 2	66.8 ± 0.5**	30 ± 3	46 ± 2	37 ± 1	30.5 ± 0.2	27 ± 3	27 ± 6
4	63.1 ± 0.2	60.5 ± 0.3**						

\* The numbers shown in the table are average recovery ± standard deviation calculated by propagation of errors.

\*\* There are no significant differences between recoveries and deliveries in vitro at  $p < 0.01$  by Student's t- test.

**F2**

In vivo delivery characteristics of a probe implanted in the thigh muscle of rat 3.

**Pharmacokinetics experiments**

On days 1 and 5, pharmacokinetics experiments were performed by perfusing the implanted probe with Ringer's solution at 2  $\mu\text{L}/\text{min}$ . Samples were continuously collected over 10 min. intervals. Two blank samples were collected prior to dosing and no interferences were observed in these samples. A dose of acetaminophen (25 mg/kg) in 1 mL saline solution was administered into the femoral vein. Dialysate samples were collected for 4 hours after dosing at 10 min. intervals. Concentrations of acetaminophen were calculated by determining the dialysate concentration from a standard curve and corrected by using the in vivo deliv-

ery of the dialysis probe. Pharmacokinetics Analysis (PKA) software from BAS was used to convert the original chromatographic data to concentrations and then plot and fit the pharmacokinetic data curves.

**Results and Discussion**

In vitro recoveries and deliveries are shown in **T1**. None of the probes showed a significant difference between recovery and delivery in vitro. Theoretically, in a stirred solution around the probe at constant temperature and perfusion flow rate, recovery and delivery of a given compound should be the same. Our results supported this theory and are in agreement with previously reported findings (18).

We used the agreement of recovery and delivery in vitro as an initial evaluation of the reliability of the probe.

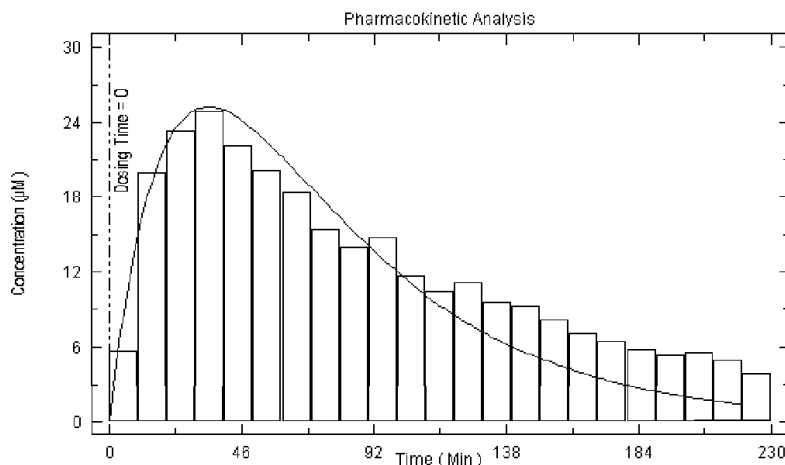
**T1** also includes the daily averages for delivery in vivo. These were lower than in vitro deliveries for the same probe. That in vivo delivery is different from in vitro delivery is not unexpected, since it is well known that in vivo recovery and delivery depends mainly on the properties of the medium surrounding the probe (19).

Several approaches have been used to determine in vivo recovery (20). In vivo delivery of the analyte of interest has also been validated as a means of determining in vivo recovery in muscle (21). Using in vivo delivery to correct the dialysate concentrations is more accurate than using in vitro recovery. Our results showed that in vivo deliveries changed from day to day in the same animal. Therefore, the daily in vivo delivery value appears to be a better parameter for calculating the actual concentration of analyte in the tissue interstitial fluid. **F2** shows the daily in vivo delivery for rat 3.

No interferences were observed in the samples obtained prior to dosing. Typical concentration-time profiles of acetaminophen in muscle of rat 3 on days 1 and 5 are shown in **F3** and **F4**, respectively. The  $t_{1/2}$  of the absorption phase was 16 min. for day 1 and 29 min. for day 5 of the experiment. The  $t_{1/2}$  of the elimination phase

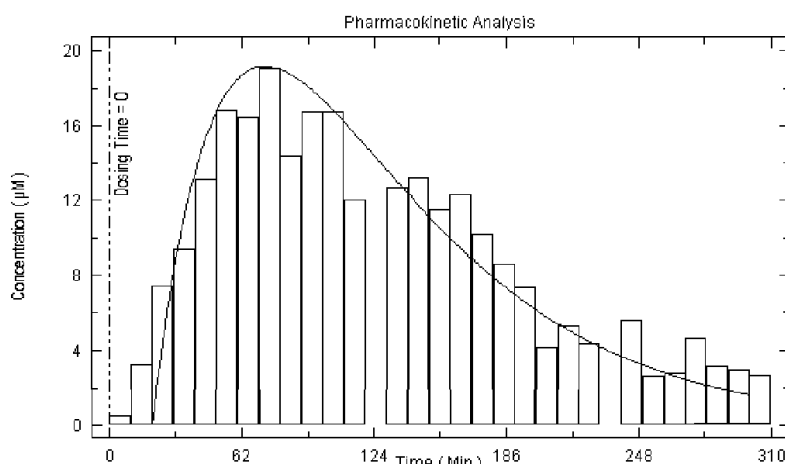
### F3

Concentration-time profile of acetaminophen in muscle. Rat 3 on day 1. ( $t_{1/2}$  for elimination = 37 min.)



### F4

Concentration-time profile of acetaminophen in muscle. Rat 3 on day 5. ( $t_{1/2}$  for elimination = 46 min.)



was 37 min. for day 1 and 46 min. for day 5 of the experiment. The peak concentration of acetaminophen in muscle dialysate was about 25  $\mu\text{M}$  on day 1 and about 19  $\mu\text{M}$  on day 5. The difference between in vivo delivery on days 1 and 5 of the experiments might be due to a change in circulation or diffusion in the tissue surrounding the probe under different circumstances (time of recovery after surgery, activity of the animal, or similar considerations). Other researchers have shown that acute inflammatory re-

sponse of the tissue to the implantation and indwelling of the probe can affect its behavior (5,21).

### Conclusions

This experiment illustrated the utility of microdialysis sampling in peripheral tissues for studying the disposition of a drug in vivo. In particular, the reliability and durability of the new linear probe were demonstrated. While absolute calibration of the microdialysis probe is very difficult in tissue, normali-

zation procedures can be used for experiment-to-experiment and time-to-time comparisons.

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