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Tech Notes

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Subcutaneous Ultrafiltration, Subcutaneous Microdialysis, Diabetes, Glucose Sampling, Surgical Implantation

Implantation of Subcutaneous UF or MD Probes in Rodents

1003

Purpose

In Vivo Ultrafiltration (UF) probes and microdialysis (MD) probes are useful tools for sampling analyte from the subcutaneous space (F1). The free concentration of many small molecules is similar in the extra-cellular fluid of the subcutaneous space and in the blood. Thus, in many cases, microdialysis and ultrafiltration provide an alternative to traditional blood sampling.

Pharmacokinetic studies and when monitoring glucose in diabetic animal models are cases on point. The advantages of these methods are that sampling is easier, they can frequently be done in an awake animal and often more samples can be obtained with greater frequency than by traditional blood sampling. For example, with a microdialysis probe you can perform a glucose tolerance test in a diabetic mouse, obtaining samples every 5 minutes. Alternately, with an ultrafiltration probe you can monitor the daily glucose levels in the diabetic mouse.

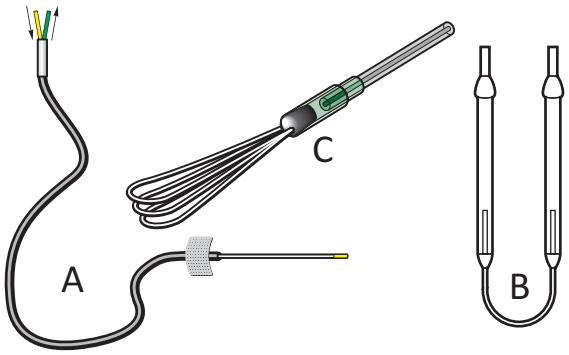

***Figure 1**. Probes for subcutaneous implantation: the IV soft flexible microdialysis probe (A), the loop microdialysis dialysis probe (B) and the in vivo ultrafiltration probe (C).*

Alternative Methods

Monitoring analyte levels in rat blood can be done by cardiac puncture, tail vein puncture or snipping off the end of the tail. Continuous monitoring in an awake rat involves cannulation of the jugular or other vasculature. Blood can be obtained from mice by cardiac puncture, from the orbital sinus or by snipping off the end of the tail. These methods all either involve more skill than is required for microdialysis or UF, or are limited by the amount of blood which can be removed without affecting the experimental status of the animal. (However, the Culex Automated Blood Sampling System is a dramatic improvement over traditional blood sampling and eliminates most of the disadvantages of blood sampling).

Available Probes

For microdialysis in the subcutaneous space the BASi IV probe is ideal. It is a flexible probe which can be used in awake animals. There is a pliable mesh on the proximal end of the probe which can be used to anchor it in place. The probe comes in two membrane lengths: 4 mm (PN MD-2305) and 10 mm (PN MD-2310). Larger loop microdialysis probes (DL probes) with 2-5 cm membranes are also available for situations in which larger probes are tolerable or desirable.

The UF probe consists of looped ultrafiltration fibers inserted into a single conduction tube. The UF-3-12 which consists of three fibers each 12 cm long (PN MF-7023), is useful in large rats. The UF-3-8 with 8 cm fibers (PN MF-7025) can be used in small rats. The three-fiber, 2 cm UF-3-2 probe (PN MF-7026) is used in mice when frequent sampling is desired over short periods of time. The one-fiber UF-1-2 probe (PN MF-7027) is used in mice when daily average samples are desired. This can be left under constant vacuum to provide continuous sampling. Smaller volumes are obtained with the UF-1 probe.

Implantation Procedure

Animal preparation:

1. Anesthetize the animal. The implantation procedure can be accomplished rapidly so a short acting anesthetic is adequate. A very useful anesthetic is a mixture of Ketamine and Xylazine. This can be easily prepared by injecting 1 mL of Xylazine (100 mg/mL) into a 10 mL bottle of Ketamine (100 mg/mL). The dose for rodents is 0.1 mL/100 g. This can be injected IP and induces surgical anesthesia within 5 min. For mice, the use of a 1/4 or 1/3 cc insulin syringe facilitates obtaining the correct dose.
2. Select an insertion site and clip hair from a 1 to 2 cm area. It is advisable to have the probe tubing exit near the base of the neck. If the probe is to remain in the animal for a long term study, it is advisable to make an initial incision and implant the probe at least 1 cm away from the tubing exit site and tunnel the tubing under the skin to the base of the neck. This gives added stability to the probe.

Depending on which type probe you will be using, follow the instructions below for implanting either UF and DL loop probes or flexible IV concentric microdialysis probes.

UF and DL Loop Probes:

1. Determine the length of the probe which will be under the skin. Clip a one cm circle of fur this distance from the insertion site. Choose a site far enough from the entry point such that the fibers and the wider non-sampling tubing near the fibers are under the skin.
2. Make a small stab incision using a #11 scalpel blade at the insertion site, the distal site and the tubing exit site at the neck (F2).

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Figure 2. *After the fur has been clipped away from the incision site, lift the skin and make a small (about 5 mm) stab incision.*

3. Place the UF probe inside the beveled introducer cannula (PN MR-5313) with the fibers at the needle end (F3). CAUTION: Be careful not to snag the fibers on the introducer bevel. Insert the introducer needle through the middle incision. Lift the skin and guide the introducer under the skin to the distal exit site.

Figure 3. *The tubing of the UF probe is inserted into the beveled end of the introducer and the fibers are pulled in until the fibers are just inside the bevel of the introducer*

4. Pull the introducer out slowly through the distal incision while holding the UF probe in place (F4). If you are working alone it may be useful to tape the probe tubing to the table to prevent pulling the probe out the distal incision along with the introducer. If the probe has been inserted too far or has been pulled out of the distal incision, gently pull it back until the junction between the large and small tubing is at the skin surface.

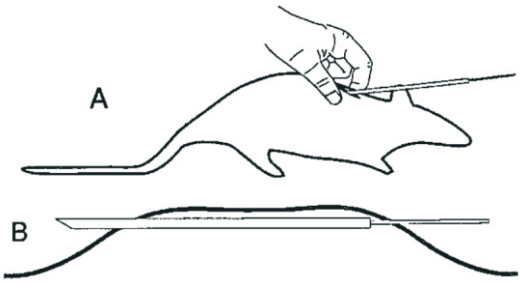

Figure 4. *Insert the introducer needle through the middle incision (A). Lift the skin and guide the introducer under the skin to the distal exit site (B).*

5. Insert the introducer into the insertion incision and externalize it from the neck incision. Thread the probe tubing into the introducer. Pull the introducer through the skin. Close all incisions with sutures. Tie the suture around the probe tubing while closing the exit incision.
6. Place a collar (MF-5371) around the neck of the rat and attach the wire tether of the Animal Sampling Caging System
7. Connect the externalized probe tubing to the Vacuum Needle Holder (MD-1322)
8. Insert a Vacutainer® (PN MF-7024) into the Vacuum Needle Holder. Fluid will likely be seen in the Vacutainer® within 15 minutes. If fluid is not seen check that the connections of the Vacuum Needle Holder are tight and try a new Vacutainer® in the Vacuum Needle Holder.
9. Tape the probe tubing and the Vacuum Needle Holder/ Vacutainer® to the flags on the wire tether.

UF and DL Probe Implantation Options:

Alternative placement procedure: In the mouse, where the introducer is only inserted a short distance, the introducer can be pulled back out of the same incision, leaving the fibers under the skin. This method avoids the distal incision. This method can also be used in the rat, but because of the greater length of fibers there is greater friction and the fibers may bend.

There are two options for placement of the DL microdialysis probes. They can either be implanted in the looped configuration, with both inlet and outlet tubing exiting from the same incision, or in the extended linear configuration, with inlet and outlet tubing exiting at different sites (F5). For awake-animal studies it is best to have both inlet and outlet tubing exiting from the same incision at the base of the neck This makes it more difficult for the animal to gain access to the tubing.

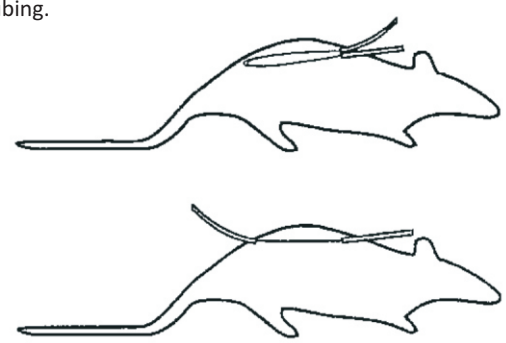

Figure 5. *DL probes can either be implanted in the looped configuration with both inlet and outlet tubing exiting form the same incision, or in a linear configuration with inlet and outlet tubing exiting at different sites.*

For placement in the linear configuration, do not place the probe in the introducer at step 3. Insert the introducer into the proximal and out of the distal incision. With both ends of the introducer externalized, thread the probe through the introducer, positioning the fibers under the skin. Pull the introducer through the skin. Close all incisions with sutures. Tie the suture around the probe tubing when dosing the incisions.

IV Style Concentric Microdialysis Probes

1. Make a small incision just large enough to accommodate the suturable mesh of the probe (about 0.5 cm) on the back or side of the animal.
2. Using a hemostat, create a track for the soft probe about 1 1/4 inch (3.2 cm) in length.
3. Insert the probe between the prongs of the hemostat (F6). Remove the hemostat, leaving the probe in place.

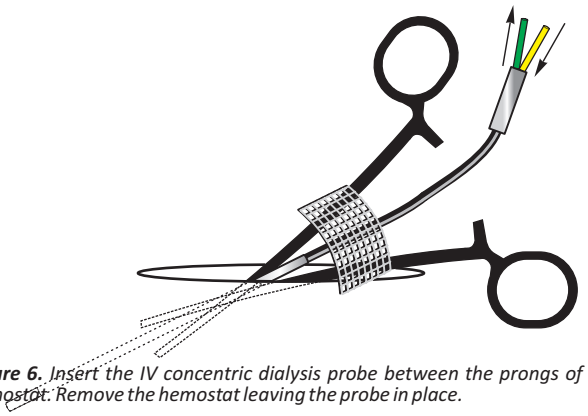

Figure 6. *Insert the IV concentric dialysis probe between the prongs of the hemostat. Remove the hemostat leaving the probe in place.*

4. Suture the flanges of the probe to the underlying tissue (F7).
5. Make a second incision at least 1 cm from the first incision.

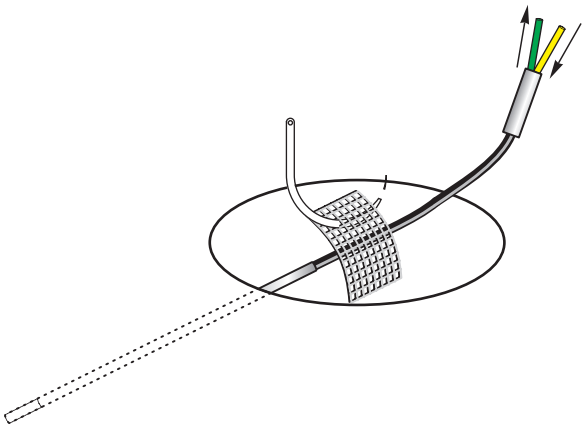

Figure 7. *Suture the mesh of the microdialysis probe to the underlying tissue.*

6. Insert the beveled introducer into one incision and out the other incision.
7. Thread the probe tubing into the cannula and pull out the introducer. The probe tubing will be exiting the skin at the second incision. Suture both incisions.
8. Place a collar (PN MF-5371) around the neck of the rat and attach the wire tether of the Animal Sampling Caging System
9. Using tubing connectors (PN MF-5163) attach sufficient tubing (PN MF-5164) to the probe to reach the top of the tether line. Tape the probe tubing to the flags of the tether wire.

Options: Steps 5 through 7 may be omitted for short-term experiments in anesthetized animals where the extra stability is not necessary.