CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Implantation of Subcutaneous Probes In Rodents

Purpose

Ultrafiltration (UF) probes and microdialysis (MD) probes are useful tools for obtaining samples from the subcutaneous space (F1). The free concentration of many small molecules is similar in the extracellular fluid of the subcutaneous space and in the blood. Therefore microdialysis and ultrafiltration provide an alternative to blood in many cases such as pharmacokinetic studies and monitoring of glucose in diabetic animal models. The advantages of these methods are that sampling is easier, they can be done in an awake animal and many more samples can be obtained with greater frequency than by blood sampling. For example, one can perform a glucose tolerance test in a diabetic mouse, obtaining samples every 5 minutes, if desired, with a microdialysis probe; or one can monitor the daily glucose levels in a mouse treated with streptozotocin using an ultrafiltration probe.

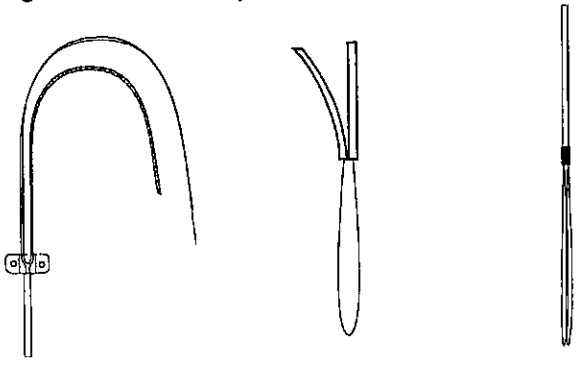

Figure 1. Probes for subcutaneous implantation: the CMA/20 soft flexible microdialysis probe (A), the loop dialysis probe (B) and the ultrafiltration probe (C).

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 vein. 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 MD or UF, or are limited by the amount of blood which can be removed without affecting the experimental status of the animal.

Available Probes

For MD in the subcutaneous space the CMA/20 probe is ideal. It is a soft and flexible probe which can be used in awake animals. There are two flanges on the probe which can be used to anchor it in place. The probe comes in two membrane sizes: 4 mm (PN MF-5388) and 10 mm (PN MF-5289). Larger loop dialysis probes (DL probes) with 2-5 cm membranes are also available from BAS for situations in which larger probes are tolerable or desirable.

The UF probe consists of looped UF 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.

Follow the instructions for either UF and DL loop probes or soft concentric MD 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 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).

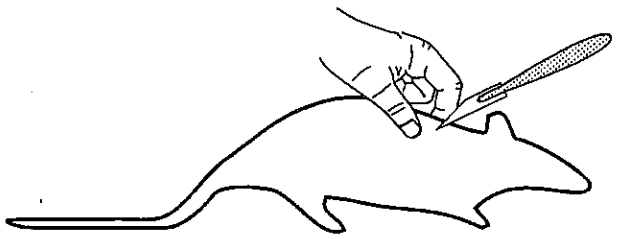

Figure 2. After the fur has been clipped away from the incision site, lift the skin and make a small (about 5mm) stab incision.

3. Place the UF probe inside the beveled introducer cannula (PN MF-7021) 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.

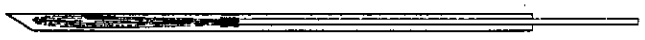

Figure 3. The tubing of the UF probe is inserted into the beveled end of the introducer and the fibers are pulled in until the fibers are just inside the bevel of the introducer

4. Pull the introducer out slowly through the distal incision while holding the UF probe in place (F4). If you are working alone it is 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.

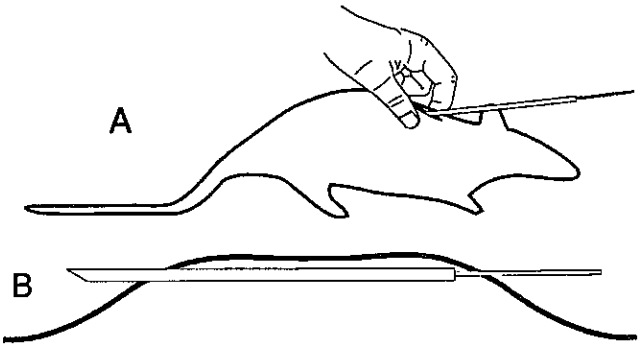

Figure 4. Insert the introducer needle through the middle incision (A). Lift the skin and guide the introducer under the skin to the distal exit site (B).

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 in closing the exit incision.

6. Place a collar (MF-5371) around the neck of the rat and attach the wire tether of the CMA/120 awake animal system (PN MF-5172).

7. Attach the hub assembly (PN MF-7021) (F5). Remove the fingertight fitting from the hub assembly. Slide the probe tubing through the fingertight fitting, barrel and needle. (It may be necessary to disassemble the needle and barrel and slide the tubing through each individually). Advance the probe tubing until it is just visible at the needle end. Tighten the connections. For hub reuse see note below.

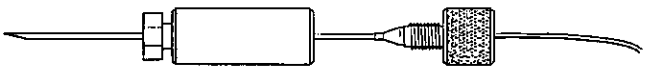

Figure 5. To attach the hub to the UF probe, slide the probe tubing through the fingertight fitting, barrel and needle until the tubing is just visible at the needle end. Pull the tubing back slightly. Tighten the fingertight fitting.

8. Insert the needle into a Vacutainer® ...fluid should be seen within 15 minutes. If fluid is not seen check that the connections of the hub are tight and insert the hub into a new Vacutainer®.

9. Tape the probe tubing and the Vacutainer® to the flags on the wire tether.

Options:

For extra protection of the probe tubing, suture a Luer button (PN MF-5369) to the underlying muscle tissue at the tubing exit site in step 5 above. Thread the probe tubing through the center of the button. Attach the offset collar (PN MF-5367) to the swivel of the awake animal system. Thread the probe tubing through the flexible wire coil (PN MF-5368). Secure the coil to the button on the rat and attach it the collar on the swivel. Omit step 6. Tape the Vacutainer® directly to the coil in step 9.

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 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 configuration, with inlet and outlet tubing exiting at different sites (F6). 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.

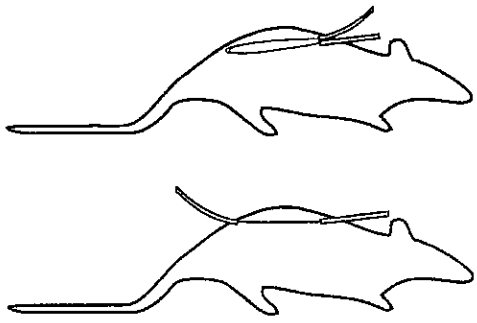

Figure 6. DL probes can either be implanted in the looped configuration with both inlet and outlet tubing exiting form the same incision, or in the extended configuration with inlet and outlet tubing exiting at different sites.

For placement in the extended 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 closing the incisions.

Note on hub re-use: Tightening of the fingertight fitting causes the internal diameter to be decreased. In order to re-use the hub, the internal diameter of the fingertight fitting must be stretched back to its original size. To do this insert the 22G rod into the fingertight so that it extends about 1/16" beyond the end. Leaving the rod in place, put the fingertight into the barrel and screw in tightly. Unscrew the fingertight, remove the rod and re-use the hub as directed above. This procedure should be performed just before use for best results.

Concentric MD Probes

1. Make a small incision just large enough to accommodate the flange 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 in length.

3. Insert the probe between the prongs of the hemostat (F7). Remove the hemostat leaving the probe in place.

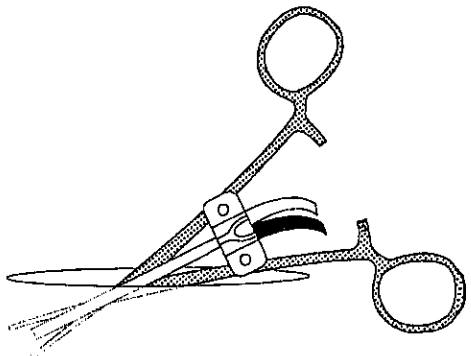

Figure 7. Insert the concentric dialysis probe (CMA/20) between the prongs of the hemostat. Remove the hemostat leaving the probe in place.

4. Suture the flanges of the probe to the underlying muscle (F8).

5. Make a second incision at least 1 cm from the first incision.

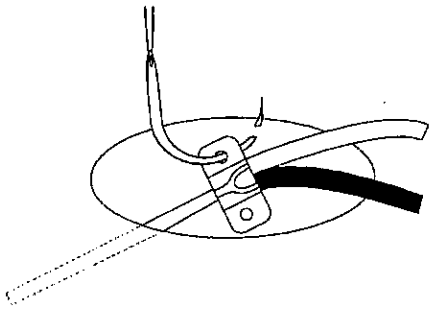

Figure 8. Suture the flanges of the CMA/20 probe to the underlying muscle.

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 (MF-5371) around the neck of the rat and attach the wire of the CMA/120 awake animal system (PN MF-5172). Using tubing connectors (PN MF- 5163) attach sufficient tubing (PN MF-5164) to the probe to reach the swivel. Tape the probe tubing to the flags of the attachment wire.

Options:

Steps 5 through 7 may be omitted for short-term experiments where the extra stability is unnecessary.

For extra protection of the tubing, suture a Luer button (PN MF-5369) to the underlying muscle tissue at step 7 above. Thread the probe tubing through the center of the button. Attach the offset collar (PN MF-5367) to the swivel of the awake animal system. Thread the probe tubing through the flexible wire coil (PN MF-5368). Secure the coil to the button on the rat and attach it the collar on the swivel. Omit step 8.