LM Linear Probes

User's Guide

02/06 rev.
A-1340

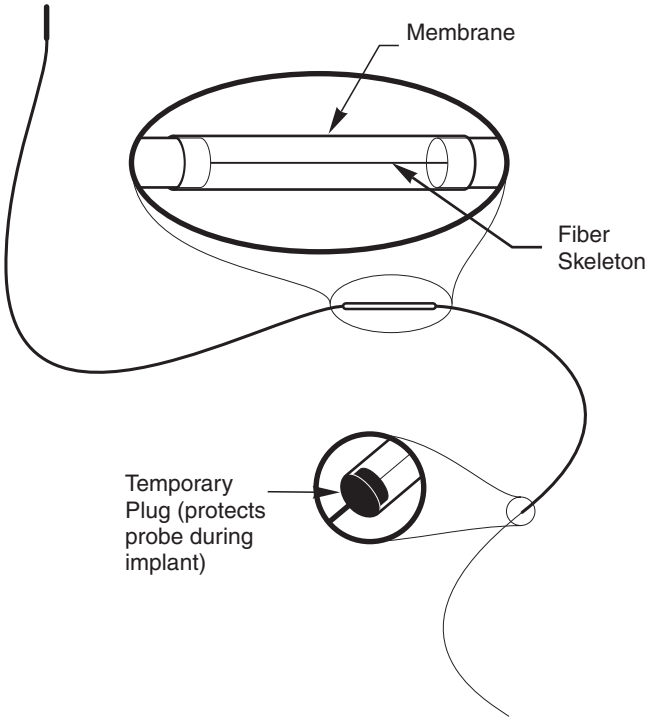
Introduction

Microdialysis sampling was originally developed in the neurosciences for use in brain tissue. The use of microdialysis sampling in pharmacokinetics research has proven to have many benefits such as more frequent data points, clean samples, no loss of body fluid and consumption of fewer experimental animals per study. The BAS linear tissue probe is suitable for in vivo sampling from peripheral tissues such as the dermis, subcutaneous tissue, muscle, liver and other organs.

The microdialysis probe consists of a short length of hollow dialysis fiber attached to narrow bore inlet and outlet tubes. An aqueous perfusion solution, which closely matches the ionic composition of the surrounding extracellular fluid, is pumped through the probe at a constant flow rate. Low molecular weight analytes diffuse in or out of the probe lumen. Large molecules such as proteins or analytes bound to protein are excluded by the membrane. Molecules entering the lumen of the probe are swept away by the perfusion fluid. This dialysate is then collected for analysis.

Design

Standard probes are available with either 10 mm or 5 mm active lengths of dialysis membrane. Long inlet and outlet tubes facilitate subcutaneous externalization of the probes when used in awake animals. The fiber skeleton assists implantation and strengthens the probe. A plug seals the end of the probe from which the fiber skeleton extends. This plug keeps body fluids from entering the probe during surgery. It must be cut off before the probe is connected to a syringe pump and perfused.



Probe Preparation

Pores within the dialysis membrane are coated with a protective layer of glycerol. Until removed, glycerol may interfere with assay results or affect recovery. For in vivo studies, the probe is usually implanted without flushing. Glycerol will be metabolized by the tissue during normal recovery (usually within a few days after the implant surgery). Pretreatment of the probe is not recommended for in vivo studies because the plug would have to be removed. Also, once wetted, the membrane becomes softer and more difficult to handle.

For in vitro studies, the temporary plug must be removed before the probe can be perfused. Place the probe in water, Ringer's solution, or artificial CSF and perfuse with the same solution at 2 µL/min for 30 minutes to remove the glycerol.

Linear microdialysis probes are guaranteed for a single use only. If used in vitro, a probe must be kept wet once wetted. Rehydration of a dried dialysis membrane will not restore function.

Probe Efficiency

Microdialysis sampling is not typically performed under equilibrium conditions, so the concentration of analyte within the lumen of the dialysis fiber will not match the concentration of analyte in the surrounding tissue. The perfusion flow rate usually sweeps the sample through the probe too rapidly for equilibrium to be established between the probe lumen and the surrounding sample matrix. The dialysate concentration of the analyte relative to the concentration in the sample matrix is termed recovery. Recovery may be thought of as a measure of probe efficiency. Among the factors which affect recovery are membrane surface area, temperature, perfusion flow rate, and nature of the analyte. The need to determine a probe's in vivo efficiency depends on the nature of the information desired from the study. Studies of endogenous compounds usually compare changes in concentration to a pre-perturbation basal level. For many pharmacokinetic applications it is not necessary to determine probe efficiency since the probe will provide reliable reflection of the analyte concentration in the extracellular fluid. If the actual tissue concentration must be obtained, in vivo calibration will be necessary.

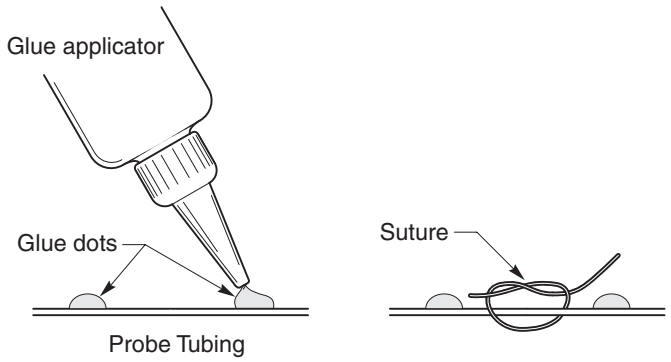
Probe Implantation in Dermis or Skeletal Muscle

If the probe has been used in vitro, or the plug and fiber extension have been removed, see the section below entitled Implanting A Probe That Has Been Used In Vitro. Otherwise, please do the following:

1. Anesthetize the animal and prepare and/or expose the target tissue. For subdermal or subcutaneus studies, this preparation may involve shaving hair and cleansing the skin over the intended implant site.
2. Thread the probe fiber into the eye of the needle.
3. Insert the needle through the tissue. The entry and exit points should be at least 4 mm further apart than the length of the probe membrane. In some cases, it may be easier to position the needle in the tissue and then thread the fiber into the needle.
4. Gently draw the needle through the tissue. Be sure the fiber remains threaded through the eye of the needle.
5. Adjust the position of the probe so the entire membrane window is centered in the tissue.

6a. Dermis: To hold the probe window in the desired position, anchor the probe to the skin using a small drop of tissue glue (MR-5314) at the entry and exit points.

6b. Muscle: To ensure that the probe window remains in position in the muscle, use tissue glue as described in 6a or apply pairs of glue dots to the probe tubing at each side. Suture the probe to the muscle between each set of glue dots.



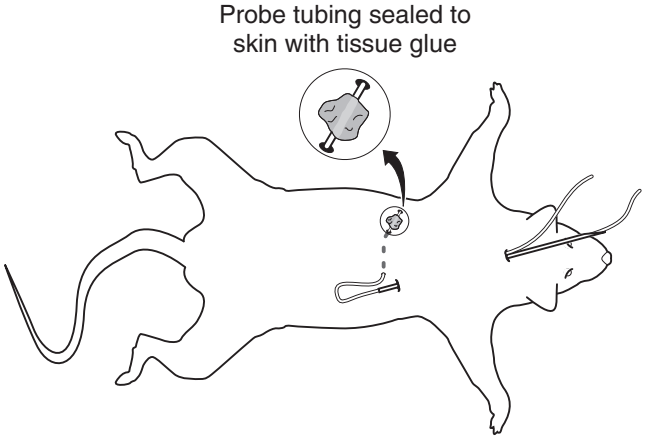
Do not tie the sutures so tightly around the probe tubing that flow is cut off or restricted.

7. If this study will be conducted in an awake and moving animal, externalize the probe tubing.

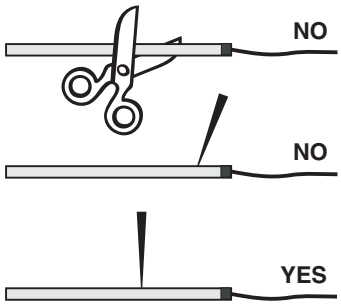
   a. For a probe implanted laterally in the skin of the rat's back, make a small lateral incision just anterior to the probe entry and exit points. Using an introducer needle (MR-5313), tunnel from an incision to an externalization site at the back of the neck. Slide the probe tubing through the introducer. Remove the introducer, leaving the probe tubing in place. Repeat for the other probe conduit. Adjust the conduits so they lay against the skin but avoid having them pulled so tight that the tubing crimps. Seal the small incisions near the probe and at the back of the neck with tissue glue.

   b. For a probe implanted in skeletal muscle, use the introducer needle to tunnel under the skin from the implantation site to the externalization site at the back of the neck. Slide the probe tubing through the introducer. Remove the introducer, leaving the probe tubing in place. Adjust the conduits as necessary so they curve smoothly away from the implantation sites without crimping. Close the incision at the back of the neck with tissue glue.

Externalizing Tubing
For Probe Implanted in Dermis



8. Using a razor blade and a blunt cut, shear the temporary glue plug from the probe. DO NOT USE SCISSORS! To be sure that you have cut away all of the plug, cut ~ 1 cm of tubing from the plug end.



   Connect the other end of the probe to a syringe pump using flanged tubing connectors (see 13 below). Begin perfusion at 1 or 2 µl/min. Allow several minutes for flow to be established through the probe. If flow does not occur, cut away an additional 5-6 mm of probe tubing. Recheck until flow is established. Turn off the pump or disconnect the probe before proceeding.

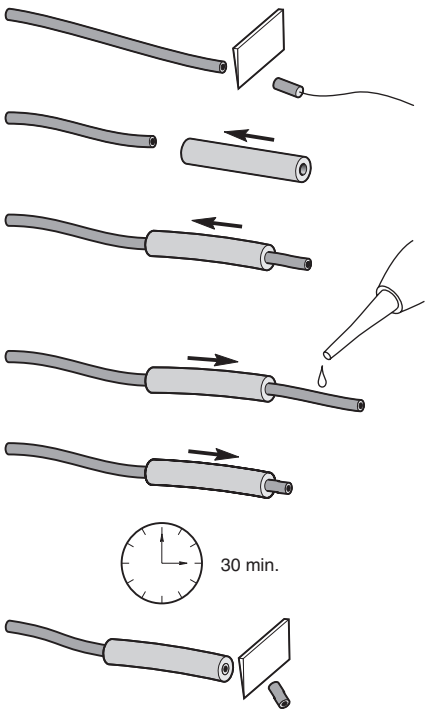
9. Remove the 1 cm long plastic connector from the center well of the probe package. Slide it over the freshly cut end of the probe, about 2 or 3 cm from the end.

10. Place a dot of MR-5314 glue on the brown probe tubing about 3 mm from the end.

    Now slide the plastic connector back over the glue and toward the cut end. Capillary action should cause the glue to run towards the connector rather than into the open end of the brown probe tubing. If using a hypodermic needle, follow instructions 3 and 4 in the previous section.

11. Let this glue joint set for at least 30 minutes. Trim off any brown probe tubing extending beyond the end of the connector. Use tubing connectors (MD-1510) to join the probe to the FEP Teflon tubing (MF-5164) which is used to make connections to syringes, fraction collectors, etc. Test perfusion flow as described in step 8, above.

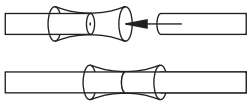
12. For studies in anesthetized animals, perfuse the probe with the preferred solution (e.g. Ringer's) for 30 to 60 minutes before beginning the experimental challenge.



13. For studies in awake animals, make the necessary connections to the tether line. If using the BeeKeeper Awake Animal System, connect the probe inlet and outlet to the liquid swivel with FEP tubing and then make connections from the swivel to the syringe pump and to the sample collection device. For the Raturn system, join the syringe pump to the probe inlet with a piece of FEP tubing fed through the central tube of the arm. Bring a second piece of FEP tubing from the probe outlet through the tube to the sample collector.

In order to minimize the dead volume of the system and avoid excessive back pressure on the probe membrane, the total tubing length connected to the probe outlet should not exceed 1 meter. For the swivel system, this is the total of tubing from probe to swivel plus that from the swivel to the sample collector.

The probe inlet and outlet tubing should be fastened to the flags on the tether line with laboratory tape. The tension should be on the tether line, not the probe tubing. However, do not leave so much slack that the animal can catch and pull or chew on the tubing.



Flanged tubing connectors join FEP tubing to inlet and outlet cannulas. Tubing should touch the cannula being joined, leaving no dead space in between.

14. Begin the perfusion at not more than 1 or 2 µL/min to establish that there is flow through the probe.

    The dead volume of the FEP tubing is 1.2 µL/100 mm. If you have used 50 cm of tubing in your system, starting at the syringe pump and ending at the fraction collector, you will have at least 6 µL of dead volume to fill (not counting the volume of swivels, probes, etc). Ata flow rate of 2 mL/min, it will take > 3 minutes before fluid will appear at the end of the last tubing segment.
15. If possible, allow the animal to awaken and recover in the awake animal bowl, with all connections to the awake animal system intact.

Implanting A Probe That Has Already Been Used In Vitro

Since the fiber extension and plug have been removed, it is necessary to use an alternate implantation technique:

1. Expose the target tissue and insert a 21 or 23 gauge hypodermic needle (at least 1 inch long) through entry and exit points in the tissue.
2. Note that the points of entry and exit should be at least 4 mm farther apart than the length of the probe membrane.
3. Thread the cut end of the brown tubing into the bevel of the needle until it exits at the hub. Gently pull the probe through until the membrane glue joint reaches the bevel. The membrane may NOT slip into the needle, but this is not necessary.
4. Holding the brown tubing and the needle hub, back the needle out, leading the membrane into place in the tissue. As soon as the membrane is fully into the tissue, release the brown tubing. Gently hold the opposite end of the probe tubing and finish extracting the needle from the tissue.
5. If necessary, gently adjust the position of the probe membrane to center it in the tissue.
6. Go back to step 6 in previous instructions. Continue to anchor probe and attach connector.

Handle a wetted probe with extra care. The membrane has been rendered more delicate by the wetting procedure.

Implanting A Probe In Liver Tissue For Awake Animal Studies

The following special items will be required for this procedure:

Quantity	*Item*
1	fine sewing needle or hypodermic needle (25 ga. X 1 or 2 inches)
2	surgical introducers (MR-5313)
1	large (14 or 16 ga.) hypodermic needle, at least 1.5 inches long
2	small glass beads (e.g. craft beads) with hole large enough to slide over connector at the end of probe

1. Shave and cleanse the back of the neck and the abdominal area.
2. At the back of the neck, make an incision only through the skin. The incision should be 1 cm long and perpendicular to the mid-line of the body.
3. Make an abdominal incision along the mid-line of the body. This incision should begin at the xiphoid process and extend about 3 cm toward the tail. The xiphoid process is a white cartilage extension of the sternum which won't be visible until the muscle wall is opened.
4. Carefully loosen the skin from the underlying muscle along the edges of the incision.
5. With the rat on its side, position the first surgical introducer by tunneling between the skin and muscle. Begin from the abdominal incision and aim up over the shoulder toward the incision at the back of the neck. Keep the pressure on the introducer toward the skin rather than the muscle. Leave the introducer in place, extending from the neck and the abdominal incision.
6. Repeat the process on the opposite side with the second introducer.
7. Place the rat on his back (the introducers will cross at the back of the neck). Using forceps with tissue grips, lift the abdominal muscle. Using small scissors, begin near the posterior end of the exposed muscle and carefully make the incision through the muscle wall. Lift the muscle away from the internal organs as you work. The placement of this incision should correspond to the one in the skin.
8. Moisten your finger tips and a cotton swab with saline solution (0.9% by weight NaCl). Use the moistened swab to gently lift the central lobe of the liver (the largest section). Carefully grasp the lobe with your finger tips. Apply just enough pressure to keep it from slipping but do not squeeze the tissue.
9. Insert the sewing needle or 25 ga hypodermic needle through the tissue, making sure that the entry and exit points are farther apart than the length of probe window and that the shaft of the needle is fully embedded in tissue. Keep in mind that the needle's pathway through the tissue defines the probe's position with respect to the tissue.

10. Thread the extension of the fiber skeleton into the eye of the needle. Pull the needle through the liver, drawing the probe into place and positioning it so that the active window is embedded and centered in the lobe. If using a hypodermic needle, follow instructions 3 and 4 in the previous section.
11. The probe can be secured in place using small beads. Thread a bead onto one end of the probe and position it about 1-2 mm from the tissue. Glue it to the probe tubing using a very small amount of tissue glue (MR-5314). Allow the glue to set, then repeat the process for the other side of the probe.

Handle the probe very carefully. Avoid tugging at the joints between the membrane and the inlet/outlet tubing. While positioning the membrane window in the tissue, don't spill glue on the liver.

12. Determine a position along the animal's side where a probe should exit the abdominal cavity. Insert a large gauge needle from between the skin and muscle into the cavity. Do not puncture any internal organs.
13. Slip the end of the probe through the needle. Remove the needle, leaving the probe tubing between the muscle and skin. Externalize the probe tubing by feeding it through the introducer and carefully removing the introducer through the back of the neck.
14. The probe tubing should make a smooth curve between the muscle and skin and out the back of the neck. Do not pull the probe tight — leave a little slack. Repeat the process on the other side.
15. Suture the incision in the abdominal muscle. Close both skin incisions using tissue glue.
16. Proceed as per step 8 in the section entitled Probe Implantation to remove the temporary glue plug and attach the connector.

Sample Handling Precautions

Microdialysis samples are particle free, protein free and ready to be injected onto a liquid chromatograph for immediate analysis. If you are loading the sample into an on-line injector, there will be no delay between the time of collection and time of analysis and there is nothing else to consider. If you are collecting samples into glass or plastic vials for later analysis, there are a few precautions that should be taken.

Microdialysates may contain the analyte you wish to study, but they will also be loaded with nutrients which have diffused out of the sample tissue (glucose, amino acids, lactate, vitamins). This makes microdialysis samples an ideal growth medium for microbes. It takes surprisingly little time for an airborne spore to land in your sample, multiply at a logarithmic rate, eat nutrients in your sample and pollute it with metabolic waste.

Retard bacterial growth by refrigerating or freezing samples as they are collected. After use, clean all parts of the system including tubing, swivels, syringes, etc. We recommend an antibacterial wash (e.g. ProClin150™ rinse, CF-2150 diluted to 0.005%) followed by thorough flushing with distilled water.

Ordering Information

MD-2000	LM-10 Linear Microdialysis Probes, 10 mm membrane window, 6/pkg.
MD-2005	LM-5 Linear Microdialysis Probes, 5 mm membrane window, 6/pkg.
CUSTOM	Any other membrane length, please inquire, allow four weeks for delivery
MR-5314	Veterinary Bonding Glue, 3 mL
MR-5313	Introducer Needle
MF-5164	FEP Teflon Tubing, 0.65 mm OD x 0.12 mm ID, 1 meter (clear)
MD-1510	Flanged Tubing Connectors (clear), 20/pkg.
MD-1001	Baby Bee Microdialysis Syringe Pump
MD-1000	Worker Bee Controller for Baby Bee
MD-1508	UniSwitch Syringe Selector: changes perfusion fluid without stopping flow
MF-5365	Surgical Instrument Kit
TM-1000	Tissue Matrix: 10 mm x 10 mm chamber for sectioning dissected tissue into slices for post-mortem histology. Other sizes and shapes (V-block, spheres) available.

Warranty

LM Linear Microdialysis Probes are warranted to be free from manufacturing defects and viable for a single use. Re-use of probes after insertion into tissue or handling during in vitro calibration studies is not guaranteed since this is wholly dependent on the skill of the individual user. BAS is liable only to the extent of replacement of defective items for claims registered within 90 days of the shipping date. BAS will not be liable for any personal injury, property damage, or consequential damages of any kind whatsoever arising from the use of the probe. This warranty does not cover damage to membranes or cannulas through improper preparation, inappropriate connections or faulty handling by the user. The foregoing warranty is in lieu of all other warranties expressed or implied but not limited to the implied warranties of merchantability and fitness for a particular purpose.

U.S. Patent 5,706,806

Bioanalytical Systems, Inc.
2701 Kent Avenue
West Lafayette, IN 47906

765-463-4527 765-497-1102 fax
www.bioanalytical.com basi@bioanalytical.com