Building Microdialysis Probes

Supplies you’ll need:

1) Silicone tubing: Polymicro Technologies (Phoenix, AZ)
   a. Part# 2000018, TSP075150, OD-150, ID-75
2) Internal infusion cannulae and guide cannulae: Plastics One (Roanoke, VA)
   a. C312I, 26ga infusion, C312G, 21ga guide
3) Spring-coated infusion connector assembly: Plastics One (Roanoke, VA)
   a. C313CS
4) Polyethylene tubing: Clay Adams (B&D); Thomas Sci. (Swedesborough, NJ)
   a. PE10 (427401), PE50 (427410)
5) Semi-permeable hollow cellulose fibers: Spectrum (Houston, TX)
   a. ID 200 µm, Molecular Weight Cut Off 18000 Dalton
6) Tubing Adaptors: CMA Microdialysis (Chemsford, MA)
   a. 340 9500 Tubing adaptors, 10/pkg
7) 2-ton epoxy, the variety that takes 8-12 hours to fully set.
8) Parafilm
9) Straight razor blade and microscope slide

Probes are built with 26-gauge Plastics One infusion cannulae, silicone tubing and polyethylene tubing, glued together with 2-ton epoxy. All microdialysis probes require an input and an output line, and a probe tip with a semi-permeable membrane at which diffusion will take place. The design allows diffusion to take place within the brain region of interest by using the ready-made infusion cannulae that fit snugly in the associated intracerebral guide cannulae, such that the length of the probe tip extends beyond the end of the guide cannulae into unadulterated brain tissue.

STEP ONE

Setting up your input & output line. You’ll need two lengths of about 40-cm PE-10 tubing (enough length so that the ends extend about 10-cm beyond the end of the spring covered connector assembly on either side (see Figure 1).

Take these lengths of PE-10 tubing and roughen 3-cm of one end on each length with fine-grained sandpaper (rub the ends back and forth on the sand paper). Then place the two rough ends next to each other (touching against the sides) against a piece of labeling tape to that the rough ends extend about 2-cm above the tape. Stick the tape against the edge of a counter or table directly in front of you so that the ends stick up from the table as shown in Figure 2.

Figure 1
Cut two lengths of silicone tubing with a razor blade against a microscope slide, one 4-cm long and one 10-cm long. Insert one of these into one of the PE-10 tubes, and the other into the other PE-10 tube, and push the silicone down about 2-cm for each. On the other end of the PE-10 tube that has the short piece of silicone tubing (below the tape in Figure 2) make a small mark with an ink marker. This will become important later when you need to know which PE-10 represents the input (marked tube) and which represents the output (unmarked tube).

Cut a small, thin piece of parafilm (about 5-mm X 3-cm), stretch slightly, and pinch around the two silicone tubes that extend above the PE-10 tubing as shown in Figure 3. The two rough ends of PE-10 tubing should be directly below the parafilm.

Mix a small amount of epoxy together and, using a small (26?) gauge needle, apply epoxy between the two PE-10 tubes and only just touching the silicone tubing extending above. Keep the majority of epoxy between the two PE-10 tubes and progressively scrape away excess moving the needle from just above the tape upwards toward the silicone tubing. But avoid getting too much glue on the silicone tubing. It should only slightly extend into the inner lumen of the PE-10 tubes, If necessary push the two silicone tubes into the PE-10 tubes slightly to push some of the glue inside the PE-10 tubes. Now wipe away all excess epoxy such that the two PE-10 tubes appear united into one larger tube (see Figure 4). It is important that this epoxy coating doesn’t extend out on the outer sides of the two PE-10 tubes, and that the bulk of the epoxy that remains is between the two PE-10 tubes along the entire length glued. Also be wary about how much epoxy enters the inner area of either PE-10 tubes as the purpose here is to join the
two PE-10 tubes together, and to join each PE-10 tube with their respectively inserted silicone tubes so that fluid can flow through the PE-10 and then through the silicone tube unobstructed and continuously. Also, the whole epoxy area must eventually fit through the inside of the connector assembly (see Figure 1).

All epoxy steps take a total of 12 hours to fully set, but after about 3 hours it is safe to go on to the next step as the epoxy will have set enough by then to maintain the shape desired (but avoid touching the epoxy area with fingers if possible until it is fully set).

**STEP TWO**

Remove the small piece of parafilm from around the two lengths of silicone tubing, as the epoxy should now hold them in place. Cut a small piece of PE-50 tubing. The length of this piece should be 1.0-cm longer than the length of the smaller bit (“this length”) of silicone tubing that extends above the epoxy coating (see Figure 5). Thus, if both the silicone tubes are threaded through this PE-50 tube, the smaller length should end up inside it with the longer length silicone extending approximately 3.0-cm on the other side. Before threading this PE-50 length, rough one end of it (about 1.0-cm) by rubbing it on fine-grained sandpaper. Then insert the two ends of silicone tubing through the PE-50 length such that the rough end eventually comes in contact with the previously epoxied PE-10 tubes.

Prepare a new small amount of epoxy and then raise the PE-50 tube slightly (~1-mm) to apply a small amount to the inside of the rough end using a 26-gauge needle. Then push the PE-50 tube back down to contact the other set epoxy shown in Figure 4. Spread a very thin coat of epoxy up around the rough end of the PE-50 length. Be sure to only spread a small amount of epoxy on the outside of the PE-50 tube as if this epoxy sets very bulky, this union won’t fit through the connector assembly shown in Figure 1. Think of this as painting a thin coating of varnish onto the outside of the PE-50 tube, rather than building a bubble of epoxy over it. After this union is finished, the final product should look something like the diagram in Figure 6. Allow these epoxy unions to fully set before moving on to Step 3.
STEP THREE

Acquire a 26-gauge internal infusion cannula (C312I from Plastics One). These cannulae are designed with a plastic collar that fits snugly into PE-50 tubing with a friction fit. In this case, the trick is to insert the silicone tubing length that extends through the PE-50 tubing into the cannula so that the plastic collar faces toward the PE-50 tubing. Then, while keeping all tubes associated (silicone tube inside and PE-50 tube outside) as STRAIGHT as possible, slowly insert the plastic collar inside the PE-50 tube. You need to be careful at this stage not to allow the silicone tubing inside to bend, particularly around the edge of the infusion cannula collar, as this will break the silicone tubing and render the apparatus useless (if this happens, start again from Step 1). It is usually best to push a little bit with thumb and pointer finger sliding upwards toward the infusion cannula, then turn the whole assembly, and push a little more the same way. Do this until the PE-50 tubing is pushed all the way up the collar to the “hilt” where it extends outward (see Figure 7). The end result should look like shown in Figure 8, with the PE-50 tubing pushed all the way up.
Having reached this step, given the extended silicone tubing is still intact; you are ready now to cut the tip length to size. You can check whether the extended silicone tubing is intact by pulling lightly on it away from the infusion cannula. If it pulls away, it was broken, and you need to start again from Step 1. The amount of silicone tubing that extends from the end of the infusion cannula outward will become the internal skeleton of the membrane tip of your microdialysis probe. Therefore, the length you cut this to will represent how long the membrane tip will be. This decision involves first determining the dorsoventral length of the brain structure you wish to dialyze from. Typically, dialysis from rat neocortex can be done with a 2-mm probe tip, while larger areas such as the rat dorsal striatum allow 4-mm to fit within their dorsoventral boundaries. Keeping the membrane tip within the boundaries of the nucleus will reinforce the argument that your neurochemical results originate from activity of said nucleus.

To make this cut, it is often wise to place the infusion cannula with silicone tubing extending from it on its side onto a glass slide on the table. Then line up vernier calipers previously set to the desired length with one edge adjacent to the end of the infusion cannula tip. Take your razor blade and push down on the silicone tubing adjacent to the other edge of the calipers, cutting off the excess silicone tubing beyond the desired length (see Figure 9).
After cutting this silicone tube to length, you are ready to epoxy the semi-permeable membrane onto the probe tip.

At this point, place the other two ends (input and output, marked and unmarked respectively) into the end of the connector assembly (shown in Figure 1) with the threaded nut (designed to mate with the guide cannula). Thread these two PE-10 tubes through this assembly until all the way through, and the only part showing at the insertion end is the top portion of the infusion cannula with the small amount of set-length silicone tubing protruding. This will allow you to perform two steps at once, namely, epoxying one end of semi-permeable cellulose membrane to the probe tip, and also installing the tubing connector onto the input (marked) PE-10 tube on the other side.

**STEP FOUR**

**GLUING THE MEMBRANE ONTO THE INFUSION CANNULA**

Attach the probe such that it stands straight up with the protruding silicone tubing pointing upward. What I use is a magnifying glass and holder device that can be found at most hardware/electronic shops with alligator clips to hold things behind the magnifying glass. This way everything is blown up and insertion of the membrane onto the tip is a snap. Cut a length of membrane tubing approximately double the length of the infusion cannula (longer than that is fine, but consider saving membrane as it can be used later to replace membrane that yields inadequate recovery after repeated use of the probe). These tubes of dialysis membrane come coated with a sugar that makes them rather stiff, which is useful because this helps make it easier to insert them onto, and into, other devices without them bunching up. Usually, I’ll cut a small, 8-cm length of silicone tubing and thread the membrane onto this first, and then use this length of tubing as a tool, to help guide the membrane onto the silicone tubing protruding from the infusion cannula tip. The idea is to thread the membrane over the silicone tubing like a sock, but into the inside of the infusion cannula as you push it downward. If using the small length of silicone tubing to do this, you pull the membrane off the tool gradually while you push it onto your probe tip (see Figures 10 & 11).
Push the membrane inside the infusion cannula such that it is likely to have filled half of the inner length of the cannula shaft. You can track this by holding the membrane with your fingers while simultaneously pulling the tool-use silicone tube upward and away from the cannula tip. This creates a space between the tool silicone tube and the probe silicone tube (indicated by the asterisk and arrow in Figure 11). Then grab both the membrane and tool by holding it lower down, and push them both down until the space between the two silicone tubes becomes minimal. By tracking the space between the silicone tubes you can keep track of how much membrane enters the inside of the infusion cannula. Mix up a small amount of epoxy. Using a 26-ga needle apply a very small amount of epoxy just above the infusion cannula tip, on the outside of the membrane. Then push the membrane down inside the cannula tip again, carrying the epoxy inside the cannula and therefore
gluing the membrane to the cannula. The important part here is that all the epoxy that forms this union be inserted INSIDE the metal shaft of the infusion cannula. You should now wipe away all the excess epoxy that falls on the outside of the metal shaft. This is when it is helpful to have a magnifying glass to look at the shaft with. Taking tiny pieces of either tissue paper, or better yet Kim-wipe brand wipes, twirling them to create thin twirled bunches, and using this to wipe away the excess epoxy is usually best. Keep wiping away the outside epoxy until there is no evidence of it on the outside of the metal. You should be able to see the gleam of light that reflects off the outside of the metal shaft unimpeded all the way up to where it joins with the membrane. In the end you may or may not be able to see the very small amount of epoxy surrounding the outside of the membrane and proceeding inside the shaft of the infusion cannula (see Figure 12).

INSTALLING THE TUBE CONNECTOR

In order to be able to attach your microdialysis probe onto typical metal connection ports of most fluid swivels (typically 23-ga), you’ll need to place a tubing connector onto the input PE-10 tube of your assembly. Unfortunately, most tube connector pieces won’t form a good enough seal on PE-10 gauge tubing, so you need to increase the diameter slightly of your input tubing so that the connector can seal to it. These tubing connectors (supply #6 above) are essentially small rubber ports that push over the tubing on either side to unite two tubes and allow solution to flow between them. Essentially, in order to create the union, you need to insert a small smoothed/cleaned piece of 26-ga needle into the inside of the PE-10 tubing. This piece need only be 3-4 mm long, but it is important that any sharp metal bits are cleared away if you use a rotary metal grinding tool to cut off this piece. I usually cut off the sharp end of the needle with a rotary metal grinding tool (Dremel drill assembly), then grab one end of the needle with a pair of curved hemostats and grind off a small piece. Then I take a thin tungsten wire (can also use the thinnest steel guitar string you can find at a cheap music shop, typically a plain A or sometimes G# string that would be about 0.011-0.012) and push it through the needle shaft, pulling back and forth to shave off any sharp bits. Then I grab this piece with the hemostats again and squirt 100% ethanol through it to clean it. Then I insert a clean wire through the piece, so that the piece of needle is about 2-cm away from one end. I insert that end into the marked PE-10 tube (remember to cut this to an adequate length to allow sufficient length to reach the port on your swivel, but not too much more, as this is where you
can cut down on dead volume). Then with tweezers I push the piece of needle inside the PE-10 tube (guided by the tungsten wire), stretching the PE-10 tube over top of the piece of needle. It may be necessary to take a new 26-ga needle and put it just barely inside the very end of your PE-10 tube and twirl, to open or “flute” out one end in preparation for insertion. You need to get the piece of needle all the way in so that it is flush with the end of the PE-10 tube then.

At this time you can take a tubing connector (preferably one that has been pre-soaked in 95% ethanol, which causes it to expand), and slide it over the needle-containing end of the input PE-10 tube. If the connector was soaked in ethanol previously it will tighten down on the end once it is allowed to dry, forming a nice seal. This will now be ready to temporarily splice to the fluid swivel port on experiment day.

STEP FIVE

GLUING THE TIP OF THE MEMBRANE

Allow the epoxy placed in Step 4 to fully set before beginning this step. This step involves placing a very small droplet of epoxy glue into the tip of the probe to glue the end of the membrane that will be farthest from the infusion cannula shaft, and therefore the part of the probe that will touch brain tissue first when inserted into the guide cannula on experiment day. To do this, we must first cut off the excess membrane that extends above the protruding silicone tubing skeleton so that only a small amount extends beyond the silicone tubing. This is best done behind the magnifying glass described for Step Four. Clean small curved & pointed surgery scissors are best to use, as they are typically sharp, yet can be handled well when everything is magnified (where small shakes are amplified). You should target about a millimeter worth of excess membrane extending beyond the silicone tubing skeleton, which you should be able to easily see through the membrane as the membranes are translucent, see Figure 13).

After the excess membrane is cut off, the goal is to place a small amount of epoxy into the short extension of membrane beyond the silicone tubing. However we obviously want to AVOID GETTING EPOXY INTO THE SILICONE TUBING as this would render the probe useless. Therefore there is a trick to moving the silicone tubing away from the membrane tip so that epoxy can be inserted without fear of blocking the silicone tubing (which will, in the end, act as the passageway for the flowing artificial CSF to exit toward the output tubing, and into the collection vial). To do this, you need to once again expose the PE-50 union onto the other side of the infusion cannula (see again Figure 8). Recall the PE-50 tubing is simply friction fitted
around the infusion cannula collar, NOT GLUED. This is important because it allows us to pull slightly back on this friction fitting. This should pull the infusion cannula shaft forward, and simultaneously pull the silicone tubing back away from the membrane tip (see Figure 14).

It is best, when doing this, to get your fingernails into the crack of the PE-50 tube and the expanded portion of the infusion cannula collar, then slowly separate. Watch the silicone tubing on the inside of the membrane moving inward, away from the membrane tip as you do this (indicated by green arrow in Figure 14). The distance to move the silicone tubing away from the membrane tip may vary with preference, but it should be moved at least 2-mm away if possible to avoid capillary action pulling the epoxy inward and blocking the silicone tube. After doing this, I usually hang the probe tip upside down, so that the membrane and skeleton point downward, prior to placing a small amount (only really visible with a magnifying glass) of epoxy into the membrane tip. Keep in mind the goal here is to fill the portion that extends beyond the silicone tube when the PE-50 friction fitting is pushed all the way up the infusion cannula collar. We need to allow this epoxy to fully set (give it 12 hours) before then pushing the PE-50 friction fit back up the plastic collar up to the “hilt” where the plastic expands outward, just as done before and shown in Figure 8. Be just as careful when doing this as you were the last time you did it to keep all the tubing behind it as STRAIGHT as possible. This will prevent the silicone tubing from breaking. If the silicone tubing breaks at this stage, you need to start again from Step 1. You will know the silicone tubing has broken if, as you push the PE-50 tube back up the collar, the silicone does not resume its previous position further down the inside of the membrane as it was before (therefore closer to the now epoxy glued tip). If everything remains intact, then you are now ready to go on to Step 6.
STEP SIX

Attach the probe assembly to your fluid swivel. It is of course best to create some sort of bracket to hold onto the plastic collar of the connector assembly rather than having the whole thing hang by the tubing connector on the input line. Once attached you should place the membrane tip into a beaker of double-distilled water such that when the assembly hangs free, the tip is fully submerged. Then you should pump double-distilled water through the probe assembly using the infusion pump you intend to use when doing microdialysis. Pump at double the rate you would normally dialyze with for at least an hour or two. This is necessary because you will wash away the sugar coating that coats the dialysis membranes and prepare your membrane for true dialysis. Without doing this step, the sugar coating may interfere with diffusion and reduce your recovery yield significantly.

MICRODIALYSIS

Doing microdialysis with this assembly is simple now, as the connector assembly is already designed to mate with the guide cannulae mentioned above (C312G). Surgical installation of these intracerebral guide cannulae should be performed as you would if you were using the cannula to make an infusion. Insert the cannula into the brain such that it sits just dorsal to the nucleus of interest. On experiment day, the microdialysis probe is inserted such that the probe’s membrane tip enters the guide cannula first, and is inserted all the way until the infusion cannula shaft connects. The connector assembly then screws onto the guide cannula. Artificial CSF flows into the input line through the tubing connector which should connect to the bottom port of the fluid swivel. The output line is then inserted into a collection vial when taking collections. The animal is then just as free to move as it would be if you were giving a drug infusion. However now you are capable of taking microdialysis collections and measuring ongoing neurochemistry changes. Alternatively, you can also infuse a drug through the input line mixed with the artificial CSF, and it will enter the brain via reverse dialysis.

MINOR ISSUES UP TO YOU

I have created holder devices made of small centrifuge tubes and large gauge needles that are cut off close to the plastic lur-loc component. Having some way of storing the probes prior to and between uses where the membrane tip is kept moist in purified water is advantageous as it keeps your probe viable for longer.
These probes can be modified in length if it is desired to have a smaller length by simply cutting down the length of both the infusion and guide cannula metal shafts prior to building them.

It is best to clean the probe assembly between uses by running double-distilled or HPLC-quality water through them to clean out the salts that may precipitate out of solution from the artificial CSF.

Rather than rebuilding the whole assembly when these probes lose recovery yield (something that should always be tracked anyway by performing simple in-vitro tests, and something that always happens eventually even to the most magical of probe designs), all one needs to do is pull the old infusion cannula shaft off at the PE-50 friction fitting. Put a new infusion cannula on. The silicone tubing should extend the same distance from this cannula as they are all made to the same length. Then, as long as the tubing has been cleaned with water and allowed to dry, a new membrane can be installed (start at Step 4). There should be no need to insert the piece of needle again into the input side, so pay attention only to the portions of the subsequent steps associated with gluing a new membrane onto the probe. In this way, one assembly can last indefinitely. I have also found that old membranes can be removed from old, used infusion cannula shafts by heating the shaft over a flame and pushing the membrane and epoxy out with a tungsten wire. Reused shafts work fine and can even save time and $ because it is then not necessary to purchase new infusion cannulae.