Inactivation Cannula Protocol

Materials

24 Gauge (G) hypodermic tube, thin wall (A-M systems, Catalog # 842400).
30 G hypodermic tube, regular wall (A-M systems, Catalog # 832000).
Polyethylene tubing, .015"x.043"x.014" (A-M systems, Catalog # 801000).
Narylene coated microelectrode (we-sense, Catalog# NW-090-145-280-60).
~0.6mm shrink tube (Advanced Polymers)
Epoxy glue
Ceramic saw/Dremel disc saw (special disc is available at dentist depot in tel-hanan, use 0.2mm disc to cut metal).
100 µl Hamilton Syringe
Syringe Pump
Permanent marker

Creating the outer Cannula

1. Cut a 35mm piece from the 24G tube for the outer Cannula using either the ceramic saw or the dremel. *Tip* – when using the ceramic saw, patiently cut with the saw until the cannula is almost cut. Do not break the cannula before.
2. Create a sharp as possible edge by filing (שיוף) the Cannula with the ceramic saw. See figure 1 for final result.
3. Cut a single piece of shrink tube about 25mm long.
4. Attach electrode to outer Cannula using the shrink tube such that the electrode is located on the long side of the Cannula tip, sticking out about 1mm (see figure 1). *Tip* – when pointing the blower on the shrink tube (in order to cause it to shrink), use the higher heat level. Do not point the blower to the shrink tube when the blower is turned on. Rather, turn off the blower and immediately point it to the shrink tube. This will prevent movement of the electrode by the strong wind from the blower.
5. *Optional* – gently create a small separation (about 10˚) of the electrode from the blunt end of the outer Cannula (best done by using blue tac) and stabilize this separation by using epoxy glue. This is done to avoid a short circuit of the electrode in case the outer isolation breaks. This stage is necessary when using glass-coated electrodes instead of nrylene-coated electrodes.

Creating the inner Cannula

6. Cut a 50mm piece from the 30G tube for the inner Cannula.
7. Gently file tip to create a uniform hole at one end. *Tip:* Use Hamilton syringe cleaning wire (inside Hamilton box) as a cleaning rod (חוטר) to open blockage.
8. Insert the inner Cannula into the outer Cannula (when the electrode is already connected).
9. Align the inner Cannula such that the tip is exactly aligned to the electrode. This can be done by *gently* pushing the inner Cannula with a finger until the finger touches the electrode.
10. The inner Cannula should stick out from the blunt end of the outer Cannula. Mark (as precisely as possible) the point where the inner Cannula leaves the blunt end of the outer Cannula.

11. Remove inner Cannula from outer Cannula.
12. Cut a piece of the polyethylene tube to required size (this depends on specific system requirements. Best to simulate exact length beforehand).
13. Put tube on inner Cannula such that the end of the tube is close to (but not on) the mark of the inner Cannula.
14. Using epoxy glue, seal connection between inner Cannula and tube. Shape the glue such that it reaches the mark and serves as a stopper for the inner Cannula.
15. When glue dries up (preferably after 24h) – check system precision. Inner Cannula tip should be aligned with electrode tip. If not, either file inner Cannula tip or epoxy glue on polyethylene tube.

Creating the inner Cannula dummy

16. Cut a ~48mm piece from the 30G tube for the inner Cannula dummy.
17. Attach a small piece (a few cm) of polyethylene tube to the edge of the dummy such that the dummy Cannula will be aligned to the sharp end of the outer Cannula (see stages 13-16).

Inactivation experiment preparation

1. Fill 1mm syringe with inactivation substance such that there are no bubbles in the syringe. Use a 25G needle.
2. Fill 100 µl Hamilton syringe with substance
3. Gently connect syringe to tube and slowly inject substance into tube. Try to visually follow fluid until it exits inner Cannula to make sure no bubbles appear.
4. Remove syringe.
5. Connect Hamilton syringe needle and gently insert fluid. A very small air bubble should appear due to the air trapped in the needle of the syringe. Keep inserting fluid until bubble is in a comfortable area to view in the tube (see running experiment section).
6. Attach Hamilton syringe to pump (if using pump).
7. Mark small lines near the area of the bubble with a 1cm distance between lines. These lines will help to track true amount of substance injected.

Running an experiment

1. Initally locate inactivation target with regular electrode.
2. Remove regular electrode and load outer Cannula to system.
3. Before inserting regular Cannula, *make sure* dura is properly open to avoid damage to Cannula and Cannula electrode.
4. Insert dummy inner Cannula into outer Cannula.
5. Slowly insert Cannula while constantly monitoring activity.
6. When reaching inactivation target, remove dummy Cannula and insert inner Cannula.
7. Activate inactivation pump (or insert inactivation fluid manually using Hamilton syringe).

8. *Importantly* – while injecting fluid, attempt to follow small air bubble in tube. If you are using the same materials as presented here, a 1 cm movement of the air bubble (marked by lines) is equivalent to injection of about 1µl of fluid.

Cleaning up

1. After removing all apparatus, clean tube and both Cannulas with distilled water.
2. Soak both Cannulas in water with detergent to remove possible blood clots (for at least half an hour)
3. Clean both Cannulas in sonicator for about half a minute each.

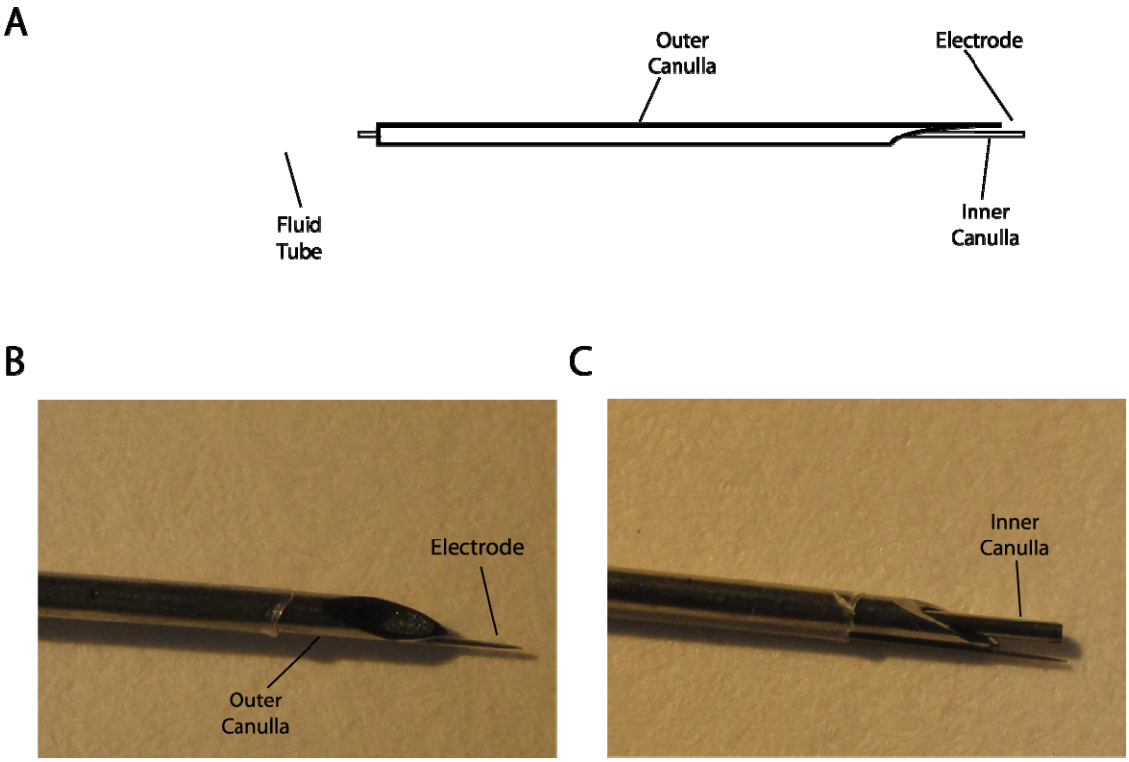

Figure 1 The inactivation Cannula system. **A** A cartoon description of the Cannula. See text for details **B**. The electrode attached to the outer Cannula attached by a shrink tube. Notice that the electrode is attached to the longer side of the Cannula. **C** The inner Cannula inside the outer Cannula.