Video Article

Fabrication of Amperometric Electrodes

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Abstract

Carbon fiber electrodes are crucial for the detection of catecholamine release from vesicles in single cells for amperometry measurements. Here, we describe the techniques needed to generate low noise (<0.5 pA) electrodes. The techniques have been modified from published descriptions by previous researchers (1,2). Electrodes are made by preparing carbon fibers and threading them individually into each capillary tube by using a vacuum with a filter to aspirate the fiber. Next, the capillary tube with fiber is pulled by an electrode puller, creating two halves, each with a fine-pointed tip. The electrodes are dipped in hot, liquid epoxy mixed with hardener to create an epoxy-glass seal. Lastly, the electrodes are placed in an oven to cure the epoxy. Careful handling of the electrodes is critical to ensure that they are made consistently and without damage. This protocol shows how to fabricate and cut amperometric electrodes for recording from single cells.

Video Link

The video component of this article can be found at https://www.jove.com/video/1040/

Protocol

Part 1: Carbon Fiber Preparation

1. Cut a bundle of carbon fibers (we use 7µm diameter) at a length that is ~1.25 X the length of the capillary pipette.
2. Remove the sizing by boiling ~100 mL of acetone in a container and adding the fibers to the hot, boiling acetone for 30 minutes or longer.
3. Remove fibers from the warm acetone, and transfer to a clean beaker with 50-100 mL of fresh acetone.
4. Dry fibers by placing the bundle on an open 150 mm Petri dish and airdry overnight.

Part 2: Fire-polishing Glass Capillary Tubes

1. Fire-polish each capillary tube (we use micro-hematocrit capillary tubes,1.5mm outer diameter, 1.3mm inner diameter, 7.5cm long) by holding the tube in the center with forceps and exposing the end to the flame.Rotate the tube about 3 times while in the flame.
2. Invert the tube and place the opposite end into the flame, again rotating 3 times.
3. Place the fire-polished tubes in a glass dish and transfer to the oven until they are ready to be filled with carbon fibers.

Part 3: Threading a Carbon Fiber into a Capillary Tube

1. Similar to the descriptions by Machado et al. (2008) and Mundroff and Wightman (2002), suck the fiber into the glass tube using plastic tubing connected to a vacuum with a filter.
2. Cut the fiber using a scalpel.Leave ¼ inch of fiber out of tube.

Part 4: Pulling Pipette-Fiber in an Electrode Puller

1. Place capillary tube into the electrode puller (we use Narishige Japan Model PP-830) and tighten the knobs to hold the capillary tube in place.
2. Manipulate the switches to execute two pulls, set at the optimal puller settings.
3. Once the pipette is pulled in half, separate the two halves by cutting the bare fiber at the center using scissors.
4. Remove each electrode half and pull on the protruding fiber to break the glass seal.Trim the fiber with scissors so that only 1/8 inch of fiber is left outside of the tube.
5. Repeat steps 1-4 until about 16 fiber-electrodes are pulled.

Part 5: Making Epoxy-Glass Seal

1. Heat a pre-made aliquot of epoxy on a hot plate set to 55°C for 6 minutes in a fume hood.
2. While epoxy is heating, wrap ~16 of the pulled electrodes into a bundle by using tape.
3. After the Epon Resin is heated for 6 minutes, add 0.7 grams xof MPDA to the hot Epon Resin (14%, w/v).Mix by swirling the vial.
4. Dip the bundle of electrodes into the hot epoxy plus hardener solution.After about 20 sec, a sufficient amount of epoxy will enter the glass tips via capillary action.
5. Undo the bundle, place electrodes onto cardboard with slots and place this tray in an oven set to 100°F.
6. After at least 48 hours, examine each electrode under a dissecting microscope.Discard electrodes that did not fill with epoxy, have a smashed tip, have more than one fiber, etc.After inspecting each electrode, place them back on the cardboard holder and back into the oven.Electrodes are usually good for about 1 month after fabrication.

Part 6:Cutting the Carbon Fiber

1. Place carbon fiber electrode carefully onto a glass slide with Teflon tape.
2. Visualize the tip under a dissecting microscope.
3. Use a clean scalpel blade to cut perpendicularly across the carbon fiber to leave a flat clean surface for placement of the carbon fiber against a cell.

Discussion

This protocol describes how to generate and cut amperometric electrodes. During amperometric recordings, a carbon fiber electrode is placed against the surface of a secretory cell. Exocytotic activity is observed as amperometric spikes, which indicates the electrochemical current caused by the transfer of electrons after oxidation of the catecholamines. With practice making and cutting amperometric electrodes, noise levels can be greatly improved over many of the commercially available electrodes.

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References

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