Protocol

Shooting DNA, Dyes, or Indicators into Tissue Slices Using the Gene Gun

Josh L. Morgan and Daniel Kerschensteiner

Imaging and reconstruction of developing neurons require cells that are labeled in a way that distinguishes them from their neighbors. This can be achieved with ballistic labeling, which refers to the delivery of a cell label by means of carrier particles (tungsten or gold) propelled from a pressurized gun. Ballistic delivery can reach many dispersed cells in one shot and can deploy a wide variety of cell markers to neurons in diverse preparations. The three most commonly used types of ballistic labels are carbocyanine dyes, dextran-conjugated fluorescent markers, and DNA plasmids. This article describes a protocol for using a Helios Gene Gun (Bio-Rad Laboratories) to inject coated particles into cells located near the surface of a tissue preparation. Shooting particles coated with carbocyanine dyes or dextran-conjugated fluorescent markers requires that a filter be placed between the gene gun and the target tissue. The filter prevents unbound dye clumps from reaching the tissue and attenuates the pressure wave reaching the tissue. DNA-coated particles can be shot without a filter if the target cells are located near enough to the surface (<20 µm deep) for the particles to penetrate using low helium pressures (35–40 psi).

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

Reagents

Media appropriate for the tissue under study

Tissue of interest

Tungsten (or gold) particles coated with dye, indicator, or DNA (Fig. 1A-D)

*For example, see **Coating Particles with Carbocyanine Dyes** (Morgan and Kerschensteiner 2011).*

Equipment

Cell culture insert (3.0-µm pore size; used as a filter) (BD Biosciences)

A filter is required for shooting particles coated with carbocyanine dyes or dextran-conjugated fluorescent markers, but not those coated with DNA (see above).

Incubator (set to the appropriate temperature and oxygen level for the tissue under study)

Gene gun (Helios; Bio-Rad Laboratories)

Tube lid (from a 15-mL tube)

Adapted from *Imaging in Developmental Biology* (ed. Sharpe and Wong). CSHL Press, Cold Spring Harbor, NY, USA, 2011.

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METHOD

Shooting Without a Filter

1. To ensure proper solenoid performance, fire the unloaded gene gun several times at 80 psi. Reduce the gas pressure to 35–40 psi before shooting tissue.
2. Drain the medium from the surface of the tissue, and place the tissue on a clean flat disposable surface such as a culture dish lid.
3. Shoot the tissue with the barrel spacer flush with the tabletop (Fig. 1E).
4. Place the tissue into the medium for 5 min to wash, and then transfer it to a fresh medium.

Proceed to Step 9.

Shooting with a Filter

5. Set the helium regulator to 80 psi, and fire the unloaded gun several times to ensure proper solenoid performance.
6. Drain the medium from the surface of the tissue, and place the tissue onto the lid of a 15-mL conical tube under the 3.0-µm pore size cell culture insert.
7. Shoot the tissue with the opening of the barrel ~1 cm from the filter (Fig. 1F).
8. Place the tissue into the medium for 5 min to wash, and then transfer it to a fresh medium.

Incubating the Treated Tissue

9. After shooting, store the tissue in an incubator set to the appropriate temperature and oxygen level for the tissue under study.
10. Image the tissues.

Adequate plasmid expression will usually be achieved after 6–24 h. Dextran-conjugated and carbocyanine dyes should fill cells within minutes.

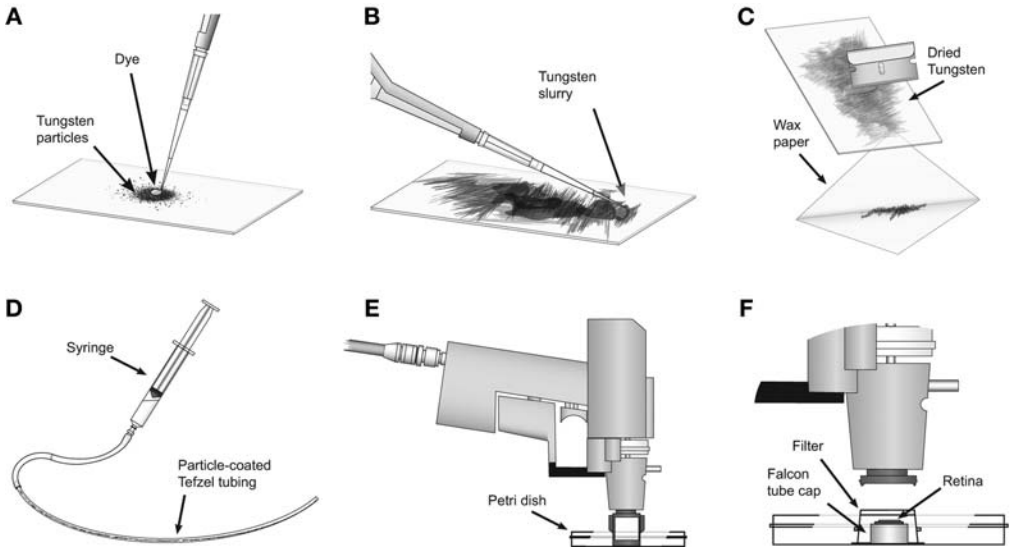

FIGURE 1. Particle coating and shooting tissue with a gene gun. (*A*) Add dye to tungsten particles on a clean glass slide. (*B*) Mix dye with tungsten, and spread across the slide to create a thin smooth film. (*C*) Gently scrape dried particles onto wax paper. (*D*) Use a 5-mL syringe to load the Tefzel tube with suspended carrier particles and to draw off the fluid once the particles have settled. (*E*) When shooting DNA-coated particles at a low pressure, the standard Helios Gene Gun barrel can be used without a filter between the gun and the tissue. (*F*) Shooting particles coated in dye or shooting DNA-coated particles at high pressures requires that a filter be placed between the gun and the tissue. The front spacer of the Helios Gene Gun barrel can be removed to achieve the proper distance between the gun and the filter (~1 cm).

DISCUSSION

The procedure described here was optimized for labeling ganglion cells in retinal flat mounts using the Helios Gene Gun. The large surface area (~35 mm^2) of this preparation and the superficial location of ganglion cell somata (<20 µm deep) make them an ideal target for ballistic labeling. However, similar protocols have been used successfully in a variety of systems, including cultured brain slices (Woods and Zito 2008).

Tissue Damage and Depth of Labeling

The two primary variables to consider when shooting are the penetration depth and the density of the carrier particles. In both cases, the number of cells labeled must be balanced against tissue damage. With no barrier between the gene gun and the targeted cells, the lowest operating pressure of the Helios Gene Gun (~35 psi) should be used. Higher pressures may be required if the target cells are covered with connective tissue, fluid, or other cells. If a filter is used to block clumps of dye, pressures in the range of 80 psi must be used. Penetration can also depend on the size of the particles. Larger particles penetrate more deeply with less pressure but are more likely to damage the cells they reach.

The deeper the carrier particles must penetrate to reach the desired cells, the greater the tissue damage that results. Ideally, the desired target cells will be within 40 µm of the surface of the tissue. Biolistic DNA transfection, in particular, is most effective when the bullet enters the cell body and is, therefore, most effective for tissues in which cell bodies are near the surface. In contrast, carbocyanine-coated particles can effectively label a cell on contact with any part of the cell membrane and, therefore, only require that target cells have a superficially located neurite. Because gold and tungsten particles damage tissue and interfere with imaging, the lowest practical density of labeling should be used. Cartridges can be cut into smaller pieces, or the volume of fluid in which carrier particles are suspended can be modified to adjust the density of shooting.

Image Acquisition

Tissue that has been labeled by ballistic delivery can be imaged using standard imaging methods. The multicolor labeling of superficially located cells lends itself to confocal imaging, although most fluorescent labels can also be efficiently (two-photon) excited with an infrared laser. A sufficiently dense layer of carrier particles will reduce image quality, but the particle density at which image quality is significantly reduced is generally greater than the density at which tissue health is compromised. However, gold particles, even at low densities, can cause other problems for two-β photon imaging. Reflection of infrared light off of gold particles can cause some photomultiplier tubes to saturate and shut off, and infrared light focused on gold particles can heat up the labeled cells. These problems can usually be overcome by limiting laser power in regions where the gold is present.

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