# Chapter 14

# Biolistic DNA Delivery to Mice with the Low Pressure Gene Gun

**Meng-Chi Yen and Ming-Derg Lai**

## Abstract

Biolistic DNA delivery is an approach to deliver plasmid to culture cells, plants, or animals. Plasmid DNA is usually transferred through bombardment of DNA-coated particles by highly pressurized gas in various kinds of delivery vehicles. The low pressure gene gun can deliver plasmid at lower pressure. Here, we describe methods of biolistic DNA delivery to mice using the low pressure gene gun.

**Key words:** Low pressure gene gun, DNA vaccine, Biolistic DNA delivery, Mouse, Naked DNA

## 1. Introduction

Biolistic DNA delivery is a physical method of gene transfer into culture cells, plants, and animals. Plasmid DNA is coated on gold particles and then is transferred to target cells or tissues by gas discharge (1). The technique can be used in vivo and in vitro. Therefore, it is widely applied in various fields, including genetic vaccination (2) and agricultural technology (3). In most of biolistic vehicles, plasmid DNA is transferred by a highly pressurized helium pulse (usually at 400 psi). In contrast, the low pressure gene gun is applicable to deliver plasmid DNA to target cells at relative low pressure (50 psi). When the helium flow travels from the inside to the outside of the spray nozzle (Fig. 1a), the gas flow accelerates to supersonic speed to deliver plasmid DNA. This vehicle has been demonstrated to transfer plasmids to cells in culture (4), to animals (5, 6), and to plant cells (7).

The other characteristic of this gene gun is to deliver gold particle-coated plasmids as well as non-particle-coated plasmids

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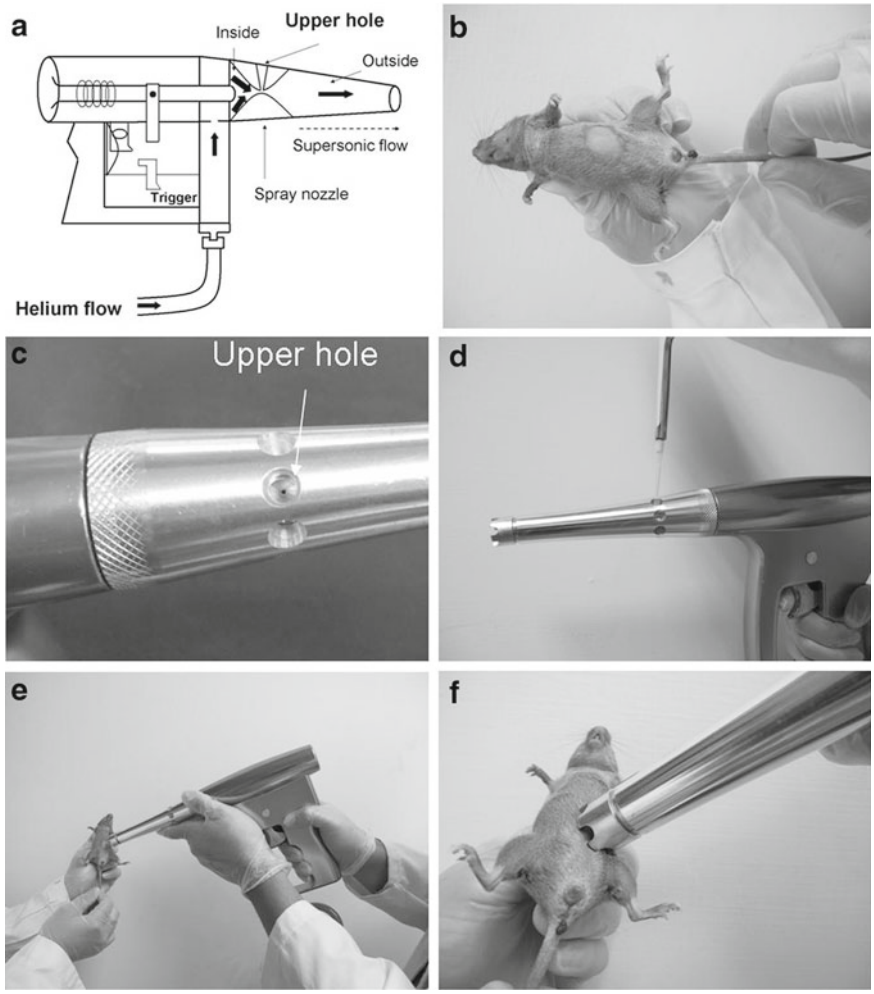


Fig. 1. Low pressure gene gun. (**a**) Schematic drawing depicting the features of the gene gun. Plasmid is loaded into the upper hole. When pressing the trigger, the helium flow travels from the inside of the spray nozzle to the outside and the pressure difference results in supersonic flow to carry particles-coated plasmids or the plasmid solution to target cells. (**b**) Shaved abdominal skin of mice. (**c**) Photograph showing the upper hole of the low pressure gene gun. (**d**) Loading of plasmid DNA into the upper hole of the low pressure gene gun. (**e**) Biolistic DNA delivery onto a mouse. One person holds the mouse and another person holds the gene gun which was loaded with plasmid DNA. (**f**) Gene gun bombardment on the shaved abdomen.

through the supersonic flow. For delivery of gold particle-coated plasmid DNA, the supersonic flow can carry the particles to penetrate through the membranes of cells. In addition, plasmids without coating on metal particles can be also delivered to target cells. The plasmid solution is sprayed out through the sprayer by pressurized gas flow. The efficacy of DNA delivery in vivo is illustrated in Fig. 2. The naked plasmid DNA delivery has been demonstrated to induce antitumor immunity in mice (8–10). Furthermore, a previous study has demonstrated that different delivery routes

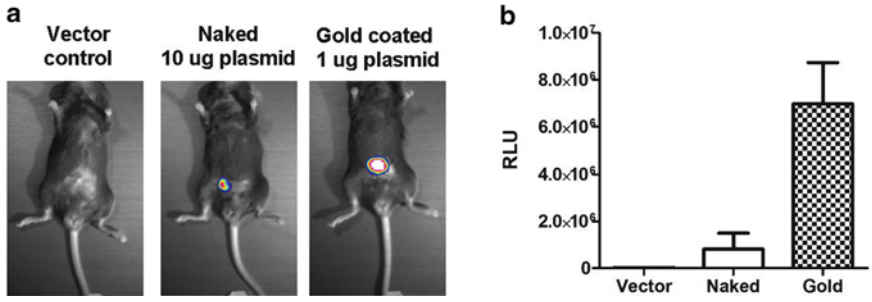


Fig. 2. Evaluation of efficiency of plasmid DNA delivery with the low pressure gene gun. (**a**) Empty plasmid vector (pGL3-Basic), 10 μg luciferase plasmid without particles and 1 μg luciferase plasmid coated on gold particles, respectively, was bombarded to C57BL/6 mice. After 48 h, luciferase activity was detected by in vivo image system. (**b**) Quantification of luciferase activity. 48 h after biolistic transfection, the skin from the plasmid-bombarded mice was homogenized. The luciferase activity of the skin lysate was determined.

result in different immune polarization (8). Th1 responses are induced by particle-free plasmid DNA delivery, whereas Th2 responses are induced by biolistic immunization using gold particle-coated plasmid DNA. It implies that the immunological polarization can be manipulated by appropriate plasmid delivery routes. Here, we describe the methods of biolistic DNA delivery with the low pressure gene gun.

## 2. Materials

### *2.1. Chemicals and Other Components*

All solutions and plasmid DNA were prepared by using ultrapure water.

1. 1.0 μm gold particles (Bio-Rad, Hercules, CA).
2. 0.05 M spermidine (Sigma-Aldrich): dissolve 0.0726 g spermidine in 10 mL sterile water and then sterilize with a 0.22 μm filter. Store at 4°C.
3. 2.5 M calcium chloride ($CaCl_2$) (Sigma-Aldrich): dissolve 2.77 g $CaCl_2$ in 10 mL sterile water. Store at 4°C.
4. Absolute ethanol (Merck).
5. Vortex mixer.
6. 1.5 mL microcentrifuge tube.
7. Ultrasonic cleaner.
8. Low pressure gene gun (BioWare Technologies Co. Ltd, Taiwan).
9. Helium gas (99.995% pure).

### 2.2. Preparation of Plasmid DNA

Prepare plasmids by using a QIAGEN endofree mega kit (Qiagen, Chatsworth, CA, USA). Adjust the stock concentration of plasmid DNA to 1 mg/mL.

## 3. Methods

### 3.1. Delivery of Plasmid DNA Precipitated onto Gold Particles

#### 3.1.1. Coating Gold Particles with Plasmid DNA

1. Estimate the amount of plasmid in each experimental group (see Note 1).
2. Weigh appropriate gold particles and add into a 1.5 mL microcentrifuge tube.
3. Add appropriate amount of plasmid DNA into the 1.5 mL microcentrifuge tube.
4. Add sterile water to 50 μL in the 1.5 mL microcentrifuge tube (see Note 2).
5. Mix the gold particles and plasmid solution by pipeting.
6. Move the tube to an ultrasonic cleaner for 3–5 s.
7. Add 75 μL of 0.05 M spermidine into the solution with continuous vortexing.
8. Add 75 μL of 2.5 M $CaCl_2$ into the solution with continuous vortexing.
9. Move the tube to the ultrasonic cleaner for 3–5 s (see Note 3).
10. Place the tube on ice for 10 min.
11. Collect the gold particle after centrifugation (10,000 × *g*) for 1 min.
12. Remove supernatant.
13. Wash gold particles with 500 μL of absolute ethanol by vortexing 3–5 s.
14. Remove ethanol after centrifugation (10,000 × *g*) for 1 min.
15. Repeat steps 13 and 14, wash gold particles twice (see Note 4).
16. Resuspend the gold particles in appropriate volume of absolute ethanol in the tube (see Note 5).
17. Pipet the ethanol and gold particles several times and aliquot 100 μL of the absolute ethanol/gold particles mixture to new tubes immediately. Each tube is placed on ice for bombardment.

#### 3.1.2. Delivery of Gold-Coated Plasmid with the Low Pressure Gene Gun

1. Shave abdominal region of mouse (see Fig. 1b).
2. Set the helium pressure at 50 psi.
3. Hold the gene gun and then load 20 μL of the mixture of absolute ethanol/gold particles to the upper pore of gene gun after pipetting (see Note 6).

4. Prepare mouse for bombardment (see Note 7).
5. Hold gene gun directly against the shaved abdomen of mouse and then press the trigger of gene gun (Fig. 1f).
6. Repeat steps 4 and 5 for five times (see Note 8).

### *3.2. Delivery of Naked Plasmid DNA Without Particles*

1. Shave abdominal region of mice.
2. Set the helium pressure at 50 psi
3. Dilute stock plasmid DNA with sterile water to the appropriate concentration (see Note 9).
4. Hold the gene gun and load 20 μL of diluted plasmid solution to the upper pore of the gene gun (see Note 6).
5. Prepare mouse for bombardment (see Note 7).
6. Hold the gene gun directly against the shaved abdomen of the mouse and then press the trigger of the gene gun (see Note 10).

## 4. Notes

1. Each mouse is bombarded with 1 μg plasmid. In addition, the plasmid DNA and gold is at the ratio of 1 μg plasmid per mg gold particle.
2. For example, five mice are bombarded with plasmid DNA. 5 mg of gold particles is added into the tube and then 5 μL of the plasmid DNA solution (1 mg/mL) is added into the same tube. 45 μL of sterile water is added to a total volume of 50 μL.
3. The continuous vortexing is necessary when spermidine and $CaCl_2$ are added to the tube drop by drop. The sonication can avoid aggregation of gold particles.
4. To check the coating efficiency, collect the absolute ethanol-washed gold particles by centrifugation and remove absolute ethanol. Add 20–30 μL of sterile water to the gold particles to dissolve DNA and load the solution to agarose gel for electrophoresis. The visible band can be observed when a successful coating was done.
5. 1 mg of gold particles is resuspended with 100 μL of absolute ethanol. For example, add 500 μL of absolute ethanol to 5 mg of gold particles in the tube.
6. Two persons are required for gene gun bombardment. One person holds gene gun and the other one holds the mouse. In addition, the mixture of absolute ethanol/gold particles must be

loaded on the upper hole of spray nozzle and then delivered by supersonic flow. Thus, hold gene gun horizontally (Fig. 1c, d).

7. The second person holds the mouse. The abdominal region of mouse is turned towards the gene gun (Fig. 1e).
8. 100 μL mixture of absolute ethanol/gold particles is bombarded to five different regions of shaved abdominal skin. Besides, gold particles should be delivered equally in five shots. The gold particles must be mixed well to prevent the gold particles from precipitation in the bottom of the 1.5 mL tube.
9. For example, for delivery of 5 μg of plasmid DNA to a mouse the working plasmid DNA solution is diluted to 0.25 μg/μL with sterile water and then 20 μL of working plasmid solution is loaded into the gene gun and bombarded to a mouse.
10. For delivery of plasmid DNA without particles, the mouse is bombarded only once on the shaved abdominal skin.

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