

Comparison of Performance Characteristics of Different Biolistic® Devices

Erik Dunder, Tim Harris, Art Weissinger[†], Martha Wright, Janet Reed, Jan Suttie, Susan Jayne, George Jen and Gary Pace. CIBA-GEIGY Agricultural Biotechnology, P.O. Box 12257, Research Triangle Park, NC 27709 and [†]North Carolina State University, Department of Crop Science, Raleigh, NC 27695

Introduction

Use of the Biolistic technology (particle bombardment) has recently evolved to become an efficient tool for gene transfer into a wide variety of organisms (Klein, *et al.*, 1992). The Biolistic process was first described by Sanford and co-workers in 1987. Evolution of the Biolistic technology began with the gunpowder-driven dual-chambered device (Figure 1). This instrument fires a 0.22 caliber cartridge that propels a large plastic projectile loaded with millions of microscopic tungsten particles coated with biological molecules (usually DNA) on its front surface. Bombardment occurs in one chamber and the second chamber is the surge tank which functions to absorb the shock wave and gases of the gunpowder explosion. A small aperture in the stopping plate retains the plastic macrocarrier and permits the DNA-coated tungsten microcarriers to accelerate toward the target cells. After this apparatus was developed and found capable of delivering DNA into cells, John Sanford and collaborators worked with DuPont to introduce the PDS-1000 unit in 1989. This unit is also gunpowder

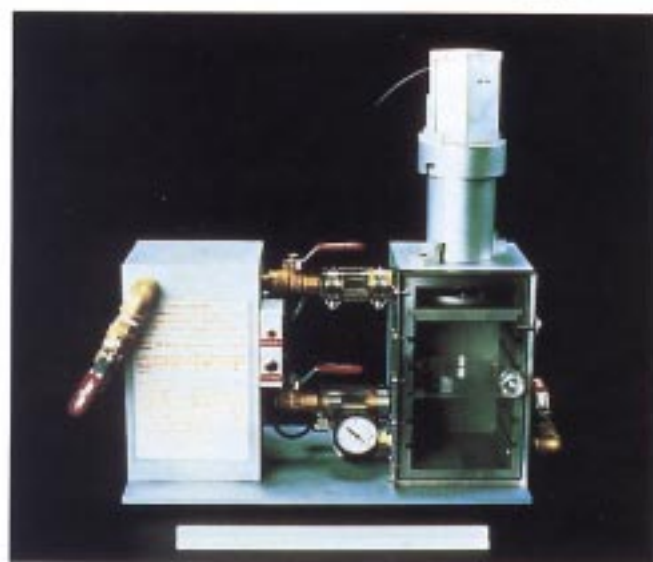


Fig. 1. Dual chamber device.



Fig. 2. PDS-1000 device.

driven, but does not require a separate surge tank (Figure 2). The most recent modification to the Biolistic technology is the helium-powered acceleration system (Sanford, *et al.*, 1991). The newest version of the Biolistic instrument, the PDS-1000/He (Figure 3), uses a helium shock wave to propel a plastic macrocarrier disk carrying DNA-coated microcarriers toward the target cells. A stopping screen retains the plastic disk, while allowing the DNA-coated microprojectiles (either tungsten or gold) to pass through and transform the target cells. The PDS-1000 gunpowder unit can easily be converted to the new helium system, the PDS-1000/He, by exchanging a few small components.

The performance characteristics of these three Biolistic instrument designs, the dual-chambered unit, the PDS-1000, and the PDS-1000/He, were compared using transient expression of a chimeric GUS gene delivered to different types of maize cells and tissue culture (stable transformation was not optimized in these studies). Shot patterns were also determined for both the gunpowder and helium devices using biotinylated DNA precipitated onto microcarriers. These patterns were compared to the distribution of GUS foci developed *in situ* to determine the most efficient delivery system for particle bombardment of target cells and tissues.



Fig. 3. PDS-1000/He device.

Methods

Bombardment Conditions

Tungsten particles (M17, -1.1μ) were used as microcarriers for both the dual-chamber and PDS-1000 gunpowder-driven units. Tungsten was coated with DNA according to the procedure of Klein *et al.* (1988a). Each gunpowder-driven bombardment used 250 μ g tungsten and 0.5 μ g DNA. Gold particles (1.6μ) were used as microcarriers for the PDS-1000/He unit and coated with DNA according to the PDS-1000/He instruction manual (DuPont/Bio-Rad). Each helium-driven bombardment used 500 μ g of gold and 0.83 μ g DNA. Target cells and tissues were held under vacuum for bombardment at 27 inches of mercury (in/Hg) and positioned 6 cm below the stopping plate/screen unless otherwise indicated. Helium rupture disks used are equivalent to 1,550 psi rupture disks and stopping screen mesh size was #24 (12 openings/cm) unless otherwise indicated. Both are commercially available from Bio-Rad Laboratories. The #10 mesh screens (4 openings/cm) and #16 mesh screens (6 openings/cm) were obtained from Small Parts, Inc. GUS expression assays were performed as described by Jefferson (1987).

Immature Embryo Targeting

Immature embryos of greenhouse-grown maize were dissected from ears approximately 10 days after pollination. Explants were placed on a Petri dish (100 x 15 mm) containing standard semi-solid medium and plated in a 4 x 4 cm target area on an 8 x 8 cm grid with each embryo on 0.5 mm centers. Approximately 2 days after bombardment, individual embryos were placed in 500 μ l of GUS histochemical reagent in multi-well plates. Blue spots were counted on each embryo after incubation for 3 days or less.

Suspension Culture Targeting

Maize suspension culture cells (100 - 200 mg) were plated onto Durapore filters and placed on a Petri dish (100 x 15 mm) containing standard semi-solid medium. Cells were harvested from the filters approximately 2 days after bombardment and incubated in GUS histochemical reagent for 3 days or less. The large number of GUS spots obtained with the PDS-1000/He required that most experiments employ a sub-sampling method of counting, based on cell spreads on grid plates.

Estimates of Variation and Statistical Analysis

The data presented comparing the different devices were collated from different experiments all using the same chimeric GUS gene (35S promoter with Adh intron #1). For these data, means and standard deviations are presented. Experiments examining different parameters for the PDS-1000/He were analyzed by ANOVA on ranked data and are presented using Duncan's Multiple Range Test (means followed by the same letter are not significantly different).

Preparations and Detection of Biotinylated DNA

Salmon sperm DNA (Sigma) was labeled with biotin using the ImmunoPure Photoactivatable Biotin (Pierce) following procedures described by the manufacturer. The level of labeling was approximately one biotin molecule per 100 to 200 nucleotide residues.

For the PDS-1000 bombardments, 5 μ g biotin-labeled DNA were precipitated onto tungsten particles according to Klein *et al.* (1988a). For the PDS-1000/He, 5 μ g of labeled DNA was precipitated onto gold particles according to the PDS-1000/He instruction manual (DuPont/Bio-Rad). One shot per membrane was used in all cases.

Each bombarded N-Hybrid membrane (Amersham) was treated by wetting successively for 45 seconds each with 1.5 M NaCl, 0.5 M NaOH, 1 M Tris-HCl [pH 8.0], 1.5 M NaCl, and 20x SSC. The processed membrane was irradiated with 2,000 μ J of short-wave UV in a Stratagene Stratalinker™ chamber. The locations of the DNA-containing particles were visualized using the BluGENE® nonradioactive nucleic acid detection system from Life Technologies, Inc., following the recommended procedures.



Fig. 4. Immature embryos bombarded with the dual-chamber device. Notice the variation in GUS spots between embryos and the damage caused by bombardment.

Results and Discussion

An example of bombarded immature embryos is shown in Figure 4. Immature embryo targeting indicated that each Biolistic device had different patterns of delivering "biologically-effective" particles. The pattern for the dual-chamber device was similar in size and shape to that observed by Klein *et al.* (1988b) using GUS delivered to tobacco suspension cells (Figure 5). The PDS-1000 produced the tightest pattern (Figure 6), whereas the dual-chamber device was intermediate. The helium device was highly effective throughout the entire 16 cm² target area (Figure 7). The density of the pattern

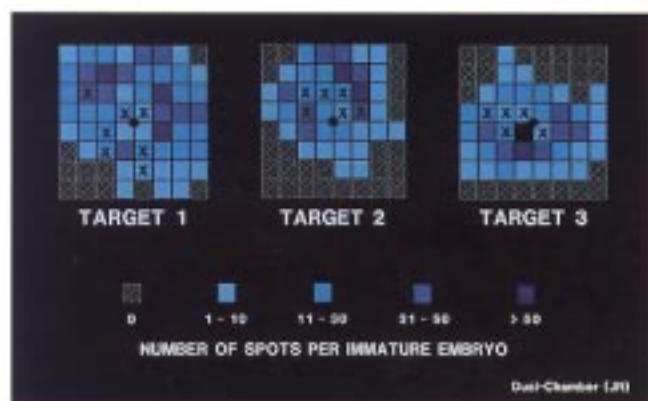


Fig. 5. Typical shot pattern for immature embryos using the dual-chamber device. X = embryo in this position damaged. Empty box indicates embryo absent from target (lost in the bombardment process).



Fig. 6. Typical shot pattern for immature embryos using the PDS-1000.

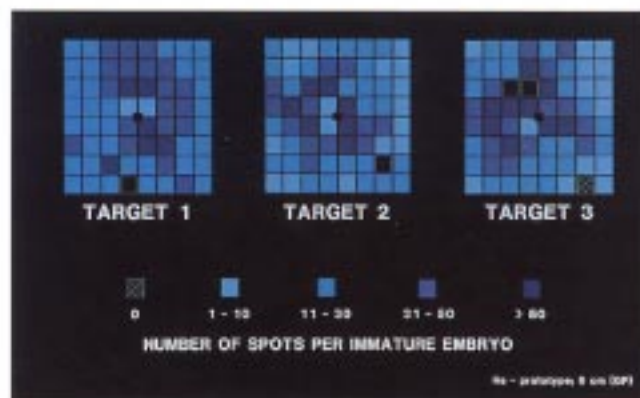


Fig. 7. Typical shot pattern for immature embryos using the PDS-1000/He. Empty box indicates embryo absent from target (lost in the bombardment process).

was ascertained by averaging both the number of spots per target area (Table 1A) and the GUS spots per embryo (Table 1B). Again, the helium device produced the most dense response for both the number of spots per target area and spots per responding embryo, with the PDS-1000 unit the least dense and the dual-chamber instrument intermediate.

Table 1. GUS spot counts on bombarded immature embryos using the three Biolistic devices

| | Dual-Chamber | PDS-1000 | PDS-1000/He |
|--|--------------|-------------|--------------|
| A. ——— GUS Spots per Target ——— | | | |
| | 1,117 | 263 | 1,858 |
| | 726 | 274 | 1,537 |
| | <u>543</u> | <u>333</u> | <u>1,574</u> |
| mean ± σ = | 725 ± 293 | 290 ± 38 | 1,656 ± 176 |
| B. ——— GUS Spots per Responding Embryo ——— | | | |
| | 20.7 | 13.2 | 29.5 |
| | 19.1 | 12.5 | 24.2 |
| | <u>13.9</u> | <u>12.8</u> | <u>25.4</u> |
| mean ± σ = | 17.9 ± 3.6 | 12.8 ± 0.4 | 26.4 ± 2.7 |

Table 2. The effect of single and multiple bombardments per target on transient GUS spot counts using suspension culture cells for the three Biolistic devices

| | Dual-Chamber | PDS-1000 | PDS-1000/He |
|---------|--------------|----------|-------------|
| 1 shot: | 197 ± 293 | 180 ± 71 | 1,553 ± 582 |
| 2 shot: | 180 ± 62 | 248 ± 51 | 1,892 ± 369 |
| 3 shot: | 249 ± 71 | 130 ± 33 | not done |

Using suspension culture cells, the number of shots delivered per target had little effect regardless of the device used (Table 2). Variation was high, which contributed to the inability to distinguish treatments. This result differs from that of Klein *et al.* (1988a), who showed with the CAT gene that increasing the number of bombardments increased transient expression in BMS suspension cells.

To further evaluate the differences between the PDS-1000 using either gunpowder or helium, a study of particle distribution was conducted. Biotin-labeled DNA was precipitated onto either tungsten (PDS-1000) or gold (PDS-1000/He) particles and delivered onto membranes. The gunpowder/tungsten combination clearly exhibited damaged filters in the center of the shot pattern (Figure 8A). This combination also produced an intense density of particles in the center. The helium/gold combination did not produce observable membrane damage (Figure 8B). Compared to the gunpowder/tungsten patterns, the helium/gold method distributed particles more evenly over a broader area with a less intense center. Shot-to-shot variation was notable for both devices.



Fig. 8. Particle distribution as revealed by biotin-labeled DNA for the PDS-1000 (A) and PDS-1000/He (B). Membranes were 6 cm from the stopping assembly.

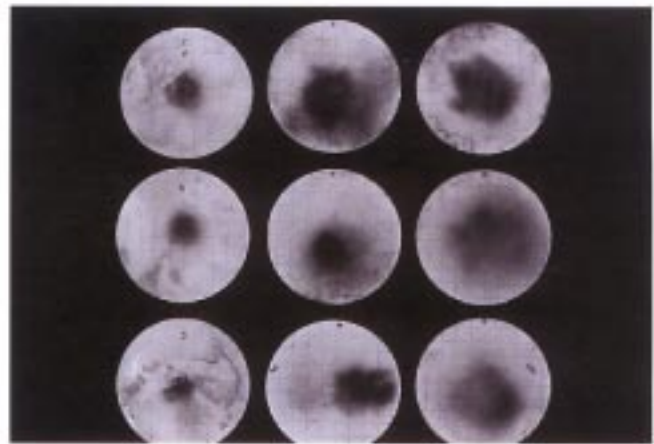


Fig. 9. Particle distribution with the PDS-1000/He at three distances (6, 9, and 12 cm) from the stopping screen, shot at 1,550 psi. Left column = 6 cm, middle = 9 cm, right = 12 cm.

The pattern of particle distribution with the PDS-1000/He changed with the distance between the stopping assembly to the target membrane. Increasing distances significantly spread out the area of greatest particle density (Figure 9). A subsequent experiment using maize suspension culture cells as a target and *in situ* development of GUS transient expression was conducted to evaluate the distribution of "biologically-effective" particles. At a distance of 12 cm it was observed that blue GUS spots appeared in a "halo" and hence did not follow the pattern observed for particle distribution (Figure 10; close-up, Figure 11). A similar halo effect was observed using a burst pressure of 1,100 psi and also observed with both gunpowder and helium units. This area of "biological effectiveness" was probably cells that were penetrated by particles and survived long enough to transiently express the GUS reporter gene.



Fig. 10. Distribution of GUS spots across a 6 cm diameter target of suspension culture cells. Delivery conditions were 1,550 psi burst pressure at a distance of 12 cm from the stopping screen.



Fig. 11. Same as Figure 10. Close-up of GUS spots on target cells.

It should be noted that similar halo effects were not observed with the immature embryo experiments used to determine target area and saturation (data not shown). This suggests that different tissues will exhibit different target characteristics when transformed with the Biolistic device.

Burst pressure of the PDS-1000/He did not seem to affect levels of transient expression in plated suspension cells (Table 3), nor did the mesh of the stopping screen (Table 4). It would appear that the use of the helium device at any pressure results in maximum levels of transient expression in maize suspension culture cells.

Considering the variability of target pattern and intensity, future comparisons of the various Biolistic devices should include target size of the experimental material. Clearly, one of the advantages of the helium device is its ability to saturate a larger area than its predecessors.

Table 3. Effect of PDS-1000/He burst pressure on the level of transient expression in plated suspension culture cells

| Helium Pressure (psi) | Number of GUS Spots | |
|-----------------------|---------------------|--------|
| | Exp. 1 | Exp. 2 |
| 800 | 678 | n.d. |
| 1,000 | 1,556 | 2,284 |
| 1,200 | 1,092 | n.d. |
| 1,400 | 1,628 | 2,434 |
| 1,800 | n.d. | 2,449 |
| 2,200 | n.d. | 2,426 |
| | (NS) | |

n.d. = Not Done

(NS) = Not a statistically significant difference

Table 4. Effect of PDS-1000/He stopping screen mesh on level of transient expression in plated suspension culture cells

| Mesh Size | Number of GUS Spots | |
|-----------|---------------------|--------|
| | Exp. 1 | Exp. 2 |
| 10 | 1,207 | 1,522 |
| 16 | 1,036 | 1,709 |
| 24 | 1,132 | 1,182 |
| | (NS) | (NS) |

(NS) = Not a statistically significant difference

Summary

Performance characteristics were determined using transient expression of a chimeric GUS gene delivered to different types of maize cells and tissue cultures. Shot patterns were also determined for the helium device using biotinylated DNA precipitated onto the gold particles. These patterns were then compared to the distribution of GUS foci developed *in situ*. Overall comparisons indicated that the helium device exhibited a 5- to 10-fold improvement in the delivery of "biologically effective" particles and a significantly greater target area compared to both of the devices based on gunpowder. The helium device was superior to the other devices in terms of effective target area and target saturation, as judged by transient expression and distribution of particles.

Bibliography

Jefferson, R. A., Assaying chimeric genes in plants: the GUS gene fusion system, *Plant Molec. Biol. Report*, **5** (4), 387-405 (1987).

Klein, T., Fromm, M., Wiessinger, A., Tomes, D., Schaaf, S., Sletten, M. and Sanford, J., Transfer of foreign gene into intact maize cells with high-velocity microprojectiles, *Proc. Natl. Acad. Sci. USA*, **85**, 4305-4309 (1988a).

Klein, T., Harper, E. C., Svab, Z., Sanford, J., Fromm, M. and Maliga, P., Stable genetic transformation of intact *Nicotiana* cells by the particle bombardment process, *Proc. Natl. Acad. Sci. USA*, **85**, 8502-8505 (1988b).

Klein, T., Arentzen, R., Lewis, P. and Fitzpatrick-McElligott, S., Transformation of microbes, plants and animals by particle bombardment, *Bio/Technol.*, **10**, 286-291 (1992).

Sanford, J. C., Devit, M. J., Russell, J. A., Smith, F. D., Harpending, P. R., Roy, M. K. and Johnston, S. A., An improved, helium-driven Biolistic device, *Technique-J. Meth. in Cell and Molec. Biol.*, **3**, 3-16 (1991).

Biolistic is a registered trademark of E.I. DuPont de Nemours and Company. The Biolistic technology is exclusively licensed by DuPont to Bio-Rad Laboratories.

