Plant Physiol. (1992) 98, 1050–1056
0032-0889/92/98/1050/07/$01.00/0

Received for publication June 28, 1991
Accepted November 6, 1991

Physical Trauma and Tungsten Toxicity Reduce the Efficiency of Biolistic Transformation[1]

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ABSTRACT

A cell suspension culture of tobacco (*Nicotiana tabacum* L.) was used as a model to study injury to cells during biolistic transformation. Lawns of cells were bombarded with tungsten particles that were coated with a plasmid containing the β-glucuronidase and the neomycin phosphotransferase II genes. When a gunpowder-driven biolistic device was used, numerous transiently expressing cells were focused around the epicenter of the blast which was manifested by a hole blown in the filter paper supporting the cells. However, transformed cells nearest the blast epicenter were injured and could not be recovered as stable transformants. The injury was primarily caused by physical trauma to the cells from gas blast and acoustic shock generated by the device. Postlaunch baffles or meshes placed in the gunpowder device reduced cell injury and increased the recovery of kanamycin-resistant colonies 3.5- and 2.5-fold, respectively. A newly developed helium-driven device was more gentle to the cells and also increased the number of transformants. Cell injury could be further moderated by using a mesh and a prelaunch baffle in the helium device. Toxicity of the tungsten microprojectiles also contributed to cell injury. Gold microprojectiles were not toxic and resulted in fourfold more kanamycin-resistant colonies than when similar quantities of similarly sized tungsten particles were used.

Biolistics (biological ballistics) is a process by which DNA or other biological materials are delivered into cells in association with high-velocity microprojectiles (17). Since its introduction in 1987, there have been numerous reports of the use of the biolistic process for the genetic transformation of the nuclear genome of plants (see reviews in refs. 14, 15, and 18). Successful uses include the production of transgenic plants in major crops such as corn (7, 8), soybean (12), and cotton (6). Biolistics has also enabled transformation of the plant chloroplast genome (2–4, 21, 23). Additionally, the biolistic process has been used to transform various microbes, including the mitochondria of yeast. Finally, it has enabled gene delivery into cultured animal cells and live animals (for refs. see ref. 18).

Although biolistics is rapidly being adopted to new applications, improvements in the technology are still needed to make it more efficient. For plant species, transient gene expression is relatively easy to achieve, but usually only a few percent of the transiently expressing cells can be recovered as stable transformants. One factor that limits the recovery of stable transformants is injury to the cells. With the commercially available GP[2]-driven biolistic device (PDS-1000, DuPont), a portion of the cells/tissues are commonly dislodged and/or killed at the epicenter of the blast, creating a central zone without transformation (1, 9, 10). It is likely that a portion of the cells outside of this zone are also injured and impaired from subsequent division and growth.

In this study, we used a tobacco cell suspension culture as a model to investigate the causes of cell injury during biolistic transformation. Our results suggest that the primary cause of cell injury is the gas blast and acoustic shock generated by the device. Additionally, the tungsten microprojectiles can themselves be toxic to cells. A new helium-driven device was found to be more gentle to the cells. Shock-attenuating mechanisms placed within the sample chamber also moderated cell injury. Gold particles could be substituted for tungsten and were not toxic.

MATERIALS AND METHODS

Plant Material and Plasmids

Cell suspension cultures of the NT1 line of tobacco (*Nicotiana tabacum* L.) were obtained from C. Paszty, University of Washington. The cultures were grown in NT1 medium (13) at 150 rpm and 24°C. Four-day-old cells, in early log phase, were used for bombardment.

Plasmids pBI426 and pBI505 were obtained from William Crosby, Plant Biotechnology Institute, Saskatoon, Saskatchewan, Canada. Plasmid pBI426 is a pUC9-based plasmid that codes for a GUS and NPTII fusion protein (5). Expression of this fusion protein is under the control of a double 35S cauliflower mosaic virus promoter plus a leader sequence from alfalfa mosaic virus. The plasmid pBI505 is similar to pBI426 but codes for GUS and not a GUS/NPTII fusion protein. Plasmid pUC118 (22), which lacks both the GUS and the NPTII genes, was used as a negative control in all experiments.

[1] This work was supported by the Cornell National Science Foundation Plant Science Center, a unit in the U.S. Department of Agriculture-Department of Energy-National Science Foundation Plant Science Centers Program and a unit of the Cornell Biotechnology Program, which is sponsored by the New York State Science and Technology Foundation, a consortium of industries, and the U.S. Army Research Office. This work was additionally supported by a grant from DuPont.

[2] Abbreviations: GP, gunpowder; GUS, β-glucuronidase; NPTII, neomycin phosphotransferase II; km^r, kanamycin resistant.

Microprojectile Bombardment Conditions

NT1 cells were collected onto filter paper discs (Whatman No. 1; 55 mm for GP device; 70 mm for helium device) by vacuum filtration in a Buchner funnel. The filter papers with the attached cells were then placed in Petri plates that contained NT1 medium solidified with Gelrite (Scott Laboratories Inc., West Warwick, RI) at a concentration of 0.8% (w/v) when the GP device was used and 0.25% (w/v) when the helium device was used.

M-10 tungsten particles, which have a mean diameter of approximately 1.0 μm (Sylvania GTE Products Corp., Towanda, PA), were coated with plasmid DNA using calcium/spermidine precipitation as previously described (4, 19). When the GP device was used, 2 μL of the DNA/particle suspension was loaded onto the macroprojectile. When the helium device was used, the DNA-coated particles were washed with 70% ethanol and again with absolute ethanol and were then resuspended in absolute ethanol. After a 3-s treatment in an ultrasonic cleaner (Branson 1200; Branson Ultrasonic Corp., Danbury, CT) to disperse the particles, 6 μL of the suspension was spread onto the center of each Kapton macroprojectile (25-mm diameter, 2 mil thick; DuPont Co.).

The GP device has been previously described (11). The target cells were placed 160 mm below the macroprojectile stopping plate.

The helium device was operated in the flying disc mode as previously described (16, 19, 23). Briefly, the sample chamber is evacuated to 0.1 atm, and the high-pressure chamber is pressurized with helium. The membrane that restrains the helium is then ruptured with a lance. The resultant shock wave of helium gas launches and accelerates the Kapton macroprojectile, which flies for a distance of either 1 or 2 cm until it is stopped by a retaining screen. The microprojectiles continue onward to penetrate the target cells. Different bombardment pressures, rupture membrane to macroprojectile distances, and macroprojectile to target cell distances were tested as described in the text.

The shock-attenuating mechanisms that were tested in the biolistic devices are detailed in Figure 1. The mesh material was either Nitex nylon monofilament with 100-μm openings (No. 3–100/47; Tetko, Inc., Elmsford, NY) or stainless steel with 94-μm openings (No. 1985–00150; Bellco Glass, Inc., Vineland, NJ).

Postbombardment Cell Handling

After bombardment, the Petri plates were placed in plastic boxes and were incubated at 24°C with indirect lighting for 2 d. Cells for histochemical GUS assays were stained with 1 mL of 5-bromo-3-chloro-3-indolyl-β-D-glucuronic acid solution (12). The cells were incubated at 37°C overnight, and the number of blue cells was counted using a dissecting microscope. Cells to be selected for kanamycin resistance were transferred on their original filter paper supports to NT1 medium with 350 mg/L of kanamycin monosulfate and 0.25% (w/v) Gelrite.

RESULTS

GP Device

When the GP device was used to bombard NT1 cells, a hole (approximately 5 mm diameter) was blown in the filter paper at the epicenter of the blast. The filter paper that surrounded the hole was bent upward, indicating that the shock rebounded after hitting the plate. Transient transformants were centered in a zone (25–35 mm in diameter) up to and around the hole in the filter paper (Fig. 2A). Bombarded cells placed on nonselective medium grew in a doughnut-shaped pattern with a distinct central zone of death (Fig. 2B), indicating that cells at the epicenter of the blast had been impaired from further growth. Km^r colonies grew only in the periphery of the blue cell zone (Fig. 3, A and C). Thus, cells in the center of the plate were apparently injured and could not be recovered as km^r colonies, even though they transiently expressed the GUS gene.

To determine whether injury to the cells was caused primarily by the force of the blast itself or by impact of the particles, lawns of cells were mock bombarded in the GP device using an unloaded macroprojectile (*i.e.* without particles or liquid). These cells also grew in a doughnut-shaped pattern (data not shown), which indicated that the blast itself was the major cause of cell injury.

Postlaunch baffles (Fig. 1A) were tested in the GP device for their ability to reduce gas and acoustic shock to the target cells. When baffles with small apertures (4 mm or less) were placed close to the cells, both injury to the cells and damage to the filter papers were significantly reduced (Fig. 3B). However, the zone of transformation was also reduced such that the total number of transformants was the same or less than when no baffle was used.

Maximal recovery of km^r colonies occurred when a postlaunch baffle with a 4-mm aperture was placed just below the stopping plate (26 mm below stopping plate, 127 mm above cells). Even though holes were still blown in the filter paper and the zone of injury was reduced only slightly (Fig. 3D), the higher percentage of conversion of transient to stable transformants (Table I) indicates that the cells were partially protected from the blast. Two baffles with progressively larger apertures (6 and 8 mm) placed below the 4-mm baffle further reduced cell injury (data not shown) but also dramatically reduced the zone and number of transformants (Table I).

When nylon or stainless steel meshes were used in the GP device, the zone of transformation was broadened. The mean number of blue cells was also higher, although not statistically significant because of higher variability (Table II). Although the force of the blast created holes in both the mesh and the filter papers supporting the cells, the zone of cell injury was slightly reduced, and there were 2.3-fold more km^r colonies (Table II).

A cushion (four layers of 55-mm filter paper on top of an open 35-mm Petri plate, leaving an air space below) placed below the cells reduced the size of the holes blown in the filter papers to <1 mm. However, the zone of cell injury was not reduced, and there was no increase in transformation (data not shown).

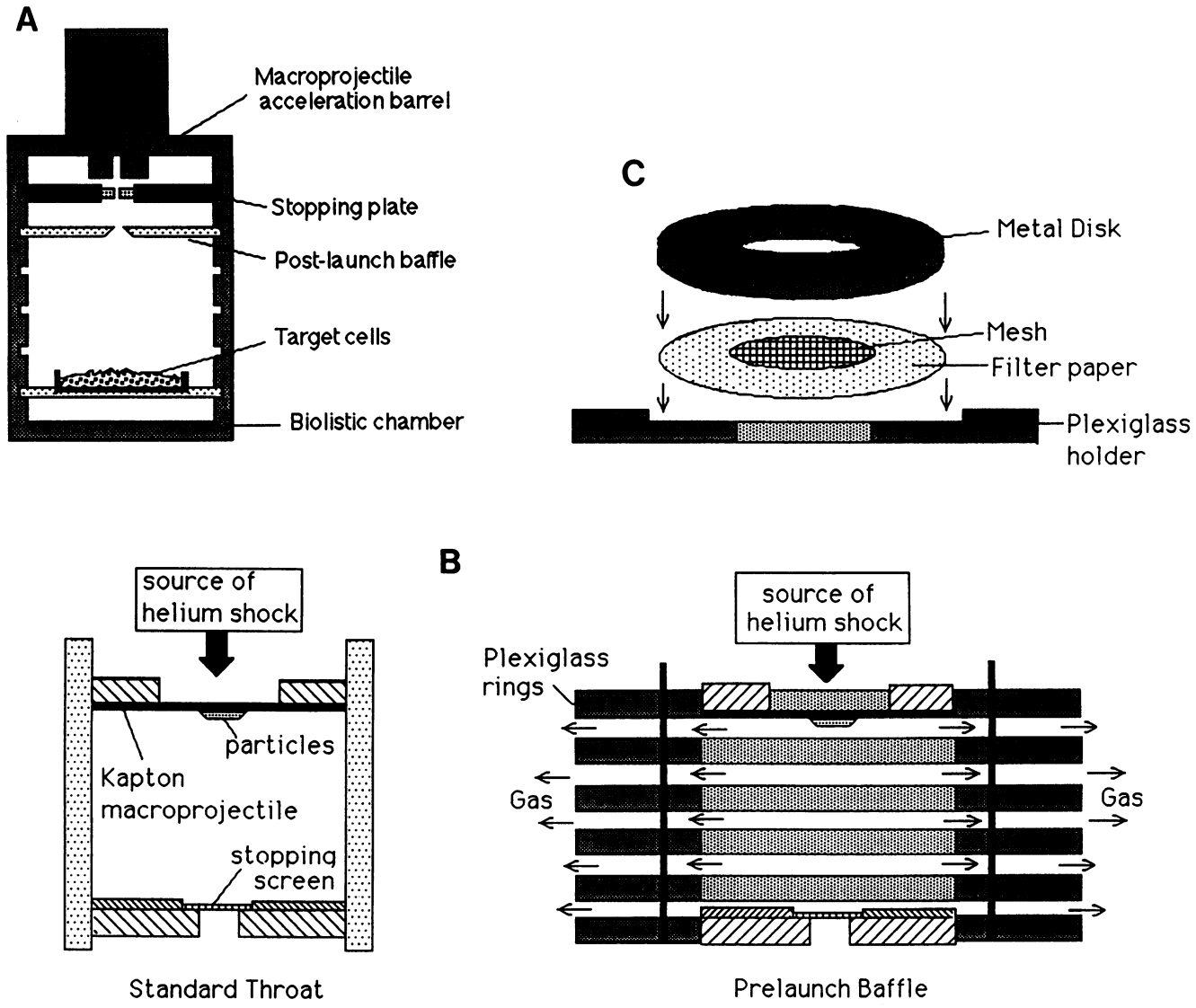

Figure 1. Shock-attenuating mechanisms. A, Position of the postlaunch baffle used in the GP device. The baffles were made from Plexiglas plates (126 × 132 × 5.5 mm thick) drilled with a single cone-shaped aperture 1, 2, 4, 6, or 8 mm in diameter. B, The prelaunch baffle that replaces the standard throat of the helium device. The baffle is made from six doughnut-shaped Plexiglas discs (each 2 mm thick, 150 mm o.d., 25 mm i.d.) spaced 1 mm apart. The macroprojectile flies through the inner portion of the baffle for a distance of 2 cm. The baffle assembly is positioned such that the distance between the rupture membrane and macroprojectile is 14 mm and the distance to the target cells is 95 mm. C, Mounting for nylon or steel mesh, to be loaded as in "A." The inner diameter of the filter paper is 25 mm.

Helium-Driven Device

When NT1 cells were bombarded with the helium device, no holes were blown in the filter paper supports, the zone of transformation was broader, and there were four- to sixfold more transformants than when the GP device was used (16; J.A. Russell, M.K. Roy, and J.C. Sanford, unpublished data). The violence of the blast could be controlled by adjusting the bombardment pressure, the distance of the target cells from the blast, and the distance between the rupture membrane and the flying disc (Figs. 4 and 5). With the proper settings (*i.e.* 115-mm target cell distance, 1000 psi, 9-mm rupture membrane to flying disc distance), cells placed on nonselective medium grew uniformly and without a visible zone of injury, demonstrating that the helium device is more gentle to the cells than is the GP device. However, when bombarded cells were placed on selective medium, km^r colonies could not be recovered from the center of the plates, even though there were large numbers of transiently expressing cells in this zone. Thus, injury from the bombardment reduces the recovery of stable transformants in the helium device as well as the GP device, even when cells show no visible injury in the absence of selection.

Two types of shock-attenuating mechanisms were tested in the helium device. The prelaunch baffle (Fig. 1B) consisted of multiple plates that replaced the throat region of the device, such that the helium shock was channeled away laterally. A nylon mesh of the type used with the GP device was also tested alone or in combination with the prelaunch baffle

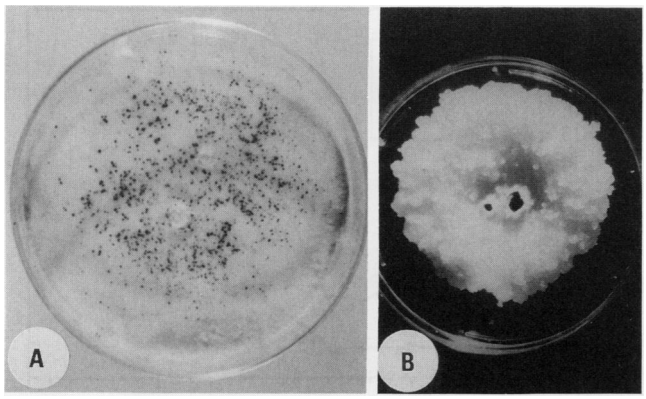

Figure 2. Bombardment of NT1 cells with pBI505-coated tungsten particles using the GP device. A, GUS-expressing blue cells (appear as black spots in this black and white photograph) 2 d after bombardment, with no apparent zone of cell injury; B, cells grown on nonselective medium for 2 weeks showing a distinct zone of death in the center.

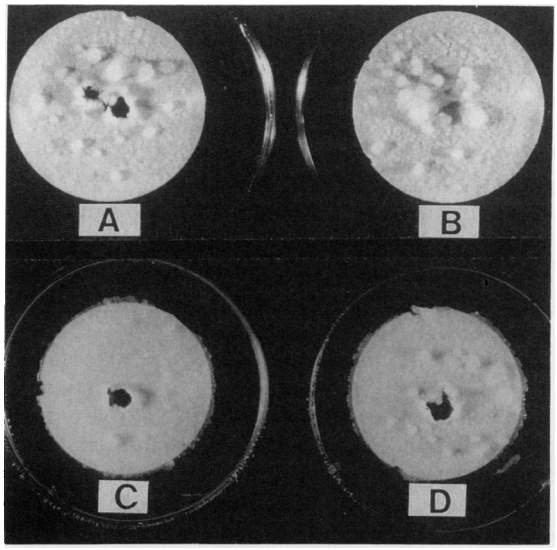

Figure 3. Effect of a postlaunch baffle with a 4-mm aperture in the GP device on the pattern of recovery of km^r colonies. Experiment 1: A, No baffle; B, baffle positioned two-thirds down the chamber; two bombardments per plate. Experiment 2: C, No baffle; D, baffle positioned just below stopping plate; one bombardment per plate. All plates were bombarded with pBI426-coated tungsten particles. Photographs were taken 6 to 8 weeks after bombardment. Filter papers are 55 mm in diameter. Note holes in the filter papers and the central zone devoid of transformants in A, C, and D.

(Table III). At 1000 psi, either the prelaunch baffle or the mesh used alone decreased injury to the cells but also reduced the zone of transformation. At 2000 psi, the zone and number of transformants with either the prelaunch baffle or the mesh was similar to the control, but cell injury was less. The highest number of km^r colonies was obtained using the baffle at 2000 psi. The combination of prelaunch baffle and mesh was the only treatment that completely eliminated the zone of cell injury, but the number of transformants was also dramatically reduced. The percentage of conversion of transient to stable transformants was not increased by any treatment.

Another method that was tested to reduce the acoustic shock to the cells was to flush the bombardment chamber with helium gas for 10 s before bombardment. A light gas such as helium should transmit a less powerful shock wave than air and might attenuate cell injury. This treatment is highly beneficial for microbial transformation (20). However, with NT1 cells, the helium flush did not reduce the zone of cell injury, and there was no difference in the number or pattern of either blue cells or km^r colonies (data not shown).

Tungsten Toxicity

Toxicity of tungsten to cells became evident to us in preliminary experiments with tobacco protoplasts. When an unloaded macroprojectile was used for bombardment, there was a large central zone of death but normal cell growth at the periphery of the plates. However, when a macroprojectile loaded with the standard mixture of tungsten particles and $CaCl_2$/spermidine was used, the protoplasts at the periphery of the plate grew abnormally and with excessive budding. This abnormal growth was absent in control plates or in plates treated with only $CaCl_2$/spermidine on the macroprojectile.

To determine whether tungsten could also be toxic to NT1 cells, M-10 particles were added to the growth medium of nonbombarded cell suspensions. In an early experiment, concentrations of 0, 100, 200, 500, and 1000 μg of M-10 particles/mL of cell culture were tested. After 1 week, cell growth in the presence of 100 μg/mL tungsten was reduced by 15% compared with the controls, and at least half of the cells were dead as could be seen by browning and collapse of the cytoplasm. At tungsten concentrations of $\geq$200 μg/mL, cell growth at 1 week was reduced 46%, and nearly all of the cells were dead. In a second experiment conducted during a 2-week period, we found that as little as 40 μg/mL of tungsten was toxic to the cells (data not shown). A single bombardment delivers approximately 400 to 500 μg of tungsten to a plate, with the highest concentration in the center. When aliquots of tungsten (40 μg or higher) were dribbled with a pipet onto lawns of NT1 cells prepared as for bombardment, cells in the

Table I. *Effect of Postlaunch Baffles in the GP Device on Transformation Rates of NT1 Tobacco Suspension Cells*

Aperture Size	No. Blue Cells/Plate[a]	No. Km^r Colonies/Plate[a]	% Conversion[b]
No baffle	456 ± 12a	4 ± 1a	0.9
4 mm[c]	325 ± 39b	15 ± 4b	4.5
4-6-8 mm[d]	83 ± 7c	3 ± 1a	3.1

[a] Mean ± 1 SE based on five replicate plates per treatment. Means within each column separated by LSD ($P = 0.05$). [b] The percentage of transiently expressing (blue) cells that could be recovered as km^r colonies. Because the blue cell assay is destructive, the percentage of conversion was estimated from parallel plates evaluated for blue cells *versus* km^r cells. [c] Single baffle placed 26 mm below the stopping plate, 127 mm above the cells. [d] Three baffles spaced 25 mm apart. The 4-mm baffle was on the top and was placed 26 mm below the stopping plate. The 8-mm baffle was on the bottom and was 127 mm above the cells.

Table II. *Effect of Meshes in the GP Device*

Mesh Type	No. Blue Cells/Plate[a]	No. Km^r Colonies/Plate[b]	% Conversion[c]
No mesh	447 ± 44a	25 ± 4a	5.6
Nylon	670 ± 210a	60 ± 12b	9.0
Stainless steel	836 ± 68a	58 ± 12b	7.0

[a] Mean ± 1 SE based on five replicate plates; no significant difference based on *F* test (P = 0.149). [b] Mean ± 1 SE based on seven replicate plates. Means separated by LSD (P = 0.05). [c] See Table I.

center did not grow, further demonstrating the role of tungsten toxicity in cell injury resulting from bombardment.

Because tungsten is classified as a "Lewis acid," we investigated the possibility that toxicity was caused by acidification of the medium. The pH of the medium decreased from 5.3 to 3.4 when 400 μg/mL of particles was added. The plant cells themselves buffered the pH somewhat (cells + 400 μg/mL tungsten = pH 4.7). When 10 mM Mes was added, the medium was fully buffered, but cell growth was still inhibited (Table IV). Therefore, the tungsten toxicity in NT1 cells is not simply due to acidification of the medium.

Gold particles (1 μm diameter, DuPont) added to the culture medium did not alter the growth of the cells or the pH. When cells were bombarded with DNA-coated gold particles, there were 997 ± 277 (mean ± SE) transient transformants per plate. This was not significantly different (P = 0.40) than when tungsten particles were used (722 ± 110 blue cells). However, the number of km^r colonies was significantly higher (P = 0.049) with gold (36 ± 12) as compared with tungsten (8 ± 1). The ratio of stable to transient transformants was also threefold higher when gold particles were used, even though the zone of cell injury was not visibly reduced.

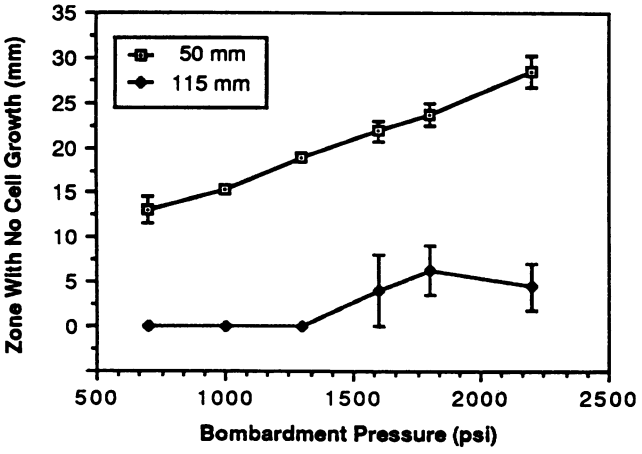

Figure 4. Effect of target cell distance and bombardment pressure in the helium device on the zone devoid of cell growth. Cells were grown on nonselective medium for 2 weeks. The rupture membrane to flying disc distance was 9 mm for all bombardments. Values represent the mean ± 1 SE of five replicate plates.

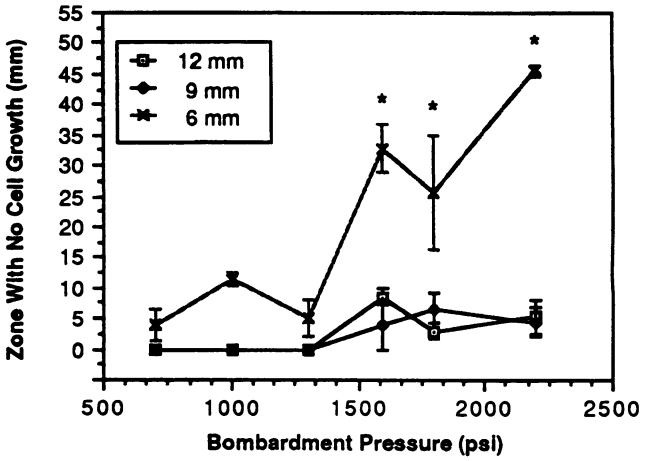

Figure 5. Effect of rupture membrane to flying disc distance and bombardment pressure in the helium device on the zone devoid of cell growth. Cells were grown on nonselective medium for 2 weeks. The target cell distance was 115 mm for all bombardments. Values represent the mean ± 1 SE of five replicate plates. *, Treatments in which the flying disc lost its integrity (ruptured) during bombardment.

DISCUSSION

For an organism to be stably transformed by the biolistic process, the microprojectiles must penetrate the cells, the DNA must be properly integrated into the genome and be expressed, and the cells must continue their growth. Generally, the conditions under which microprojectiles penetrate the largest number of cells are also the most injurious. Our results demonstrate that injured cells can transiently express a foreign gene but that such cells may not be capable of further division or growth. Thus, cell injury is one factor that limits the recovery of stable transformants.

The primary cause of injury when NT1 cells are bombarded with the GP device is physical trauma from the gas blast and acoustic shock generated by the device. The central "zone of death" indicates that cell injury is greatest at the epicenter of the blast. However, other cells on the plates are probably also traumatized. Klein *et al.* (9, 10) reported reduced rates of both transient and stable transformation at the epicenter of the blast, partially because cells were dislodged from the center of the plate. We used a smaller volume of DNA-tungsten suspension on the macroprojectile and longer target cell distances, which reduced dislodging of the cells. Armstrong and Hinchee (1) reported that the primary cause of cell injury to tobacco leaves was the impact of the tungsten particles and liquid carrier. Injury from tungsten in their report may have been more dramatic because larger M-17 particles (1.4 μm diameter) were used.

Physical trauma to the cells from the GP device can be reduced by postlaunch baffles or meshes. Postlaunch baffles, however, are inefficient because they trap many of the particles. Postlaunch baffles are most useful where only a focused zone of transformation is desired, such as for small quantities of tissue (*e.g.* meristems) or for targeting specific regions within a tissue. In the GP device, postlaunch meshes do not reduce the zone of death as much as postlaunch baffles, but

Table III. *Effect of a Prelaunch Baffle and/or Nylon Mesh in the Helium Device*

Data represent the means ± 1 SE. There were four replicate plates for the blue cell assays and five replicate plates for kanamycin selection. Means within each column that are followed by the same letter are not significantly different as determined by an LSD test at the 5% level.

Baffle Type	Bombardment Pressure	Diameter of Transformation Zone[a]	No. Blue Cells/ Plate	No. Km^r Colonies/Plate	% Conversion[b]	Zone of Cell Injury Rating[c]
	psi	*mm*				
No baffle	1000	70 ± 0c	3772 ± 653c	145 ± 18bc	3.8	4
	2000	70 ± 0c	3200 ± 790bc	124 ± 11b	3.9	5
Prelaunch	1000	40 ± 2a	1860 ± 352ab	42 ± 15a	2.2	1
	2000	70 ± 0c	4709 ± 637c	181 ± 20c	3.8	2
Mesh	1000	51 ± 7b	4273 ± 512c	161 ± 14bc	3.8	1
	2000	70 ± 0c	3063 ± 807bc	147 ± 31bc	4.8	2
Prelaunch plus mesh	1000	39 ± 2a	1063 ± 204a	17 ± 6a	1.6	0
	2000	45 ± 1a	1298 ± 169a	47 ± 11a	3.6	0

[a] Based on blue cell assay. [b] See Table I. [c] Visual rating based on size of center zone devoid of km^r colonies [0, no injury zone; 5, large (up to 50 mm) injury zone].

they do increase both the zone and number of transformants. In addition to moderating gas blast and acoustic shock, meshes also break up particle aggregates (8).

Gas blast and acoustic shock can be reduced further by using the helium device, because much of the shock is contained behind the Kapton macroprojectile. Additionally, the power of the bombardment can be adjusted for different types of tissue, and there is no damage from liquid carrier (1) because the particles are dried onto the surface of the macroprojectile. Furthermore, there is less damage to cells from impact of large aggregates of microprojectiles because the particles do not clump as much when they are suspended in absolute ethanol, are sonicated, and are then dried onto the Kapton macroprojectile. Finally, the helium device disperses the particles and the shock wave more uniformly over the target cells.

Although the helium device is more gentle than the GP device, there is still a zone of death when the cells are placed onto selective medium. Most likely, the stress of kanamycin selection compounds the stress of bombardment and impairs cell growth in the center of the plates. An alternative explanation is that there could simply be significant cell injury in the central zone from the bombardment itself that would reduce the number of km^r colonies, while overall growth in this zone on nonselective medium is not visibly impaired unless a very large fraction (>90%) of the cells are killed. Cell injury is probably caused by a "combination of insults" including vacuum, acoustic shock, gas blast, particle impact, particle penetration, tungsten toxicity, cytoplasmic leakage, and kanamycin selection. Recovery of stable transformants might be increased by waiting for a longer time before the cells are placed onto selective medium. This is impractical with NT1 cells because they rapidly overgrow the kanamycin medium but may be useful for slower growing tissues. Where target tissues are limited, it is still advisable to place such tissues in a doughnut-shaped pattern for bombardment to avoid the central zone of greatest injury.

Cell injury can be further moderated in the helium device by a prelaunch baffle and a postlaunch mesh. However, the zone of transformation is also reduced unless high pressures are used. Although the prelaunch baffle does not physically block the path of the microprojectiles, we believe the reduced zone of transformation may be caused by loss of particles laterally in the baffle or by reduced particle velocity. At any rate, the prelaunch baffle does not dramatically increase the recovery of stable transformants. The mesh does physically block the path of some of the particles before they reach the cells. It may also modify their velocity. The mesh is not as crucial in the helium device as in the GP device because particle clumping and gas blast/acoustic shock are already less severe. Shock-attenuating mechanisms in the helium device may be required for very fragile cell types or when short target cell distances are needed, such as when smaller or less dense microprojectiles are to be used. For example, the prelaunch baffle and the mesh are needed to protect leaves of tender grape seedlings when bombarding colonies of powdery

Table IV. *Effect of M-10 Tungsten Particles on pH and Cell Growth in Medium with or without Mes Buffer*

Erlenmeyer flasks (125 mL) containing 25 mL of NT1 medium (± Mes, ± M-10 particles) were inoculated with 1 mL (0.6 mL settled volume) cell suspension. The pH of the medium before autoclaving and addition of particles and cells was 5.8. The cells were incubated at 150 rpm at 24°C for 1 week. Values represent the means ± 1 SE of three replicates.

M-10 Concentration	Medium pH	Cell Growth
		mL
0 mM Mes		
0 μg/mL	5.42 ± 0.07	8.27 ± 1.52
400 μg/mL	4.45 ± 0.06	0.43 ± 0.03
10 mM Mes		
0 μg/mL	5.43 ± 0.03	10.50 ± 0.37
400 μg/mL	5.28 ± 0.17	0.50 ± 0.06

mildew on their surface (F.D. Smith, P.R. Harpending, and J.C. Sanford, unpublished data).

Toxicity of the tungsten particles also contributes to cell injury. It is not known to what extent particles within a cell cause toxicity, but they are certainly not lethal in all cases, because stable transformants can be recovered at high rates. Although our results demonstrate that high concentrations of external tungsten can be injurious, the sensitivity of cells to tungsten appears to differ among organisms. For example, *Escherichia coli* is sensitive to tungsten, whereas *Bacillus megaterium* is not, because transformed *Bacillus* colonies grow even in areas blackened by tungsten while *E. coli* will not (F.D. Smith, personal communication). Some organisms may also be adversely affected by the tungsten-induced acidification of the medium. In those cases, buffers such as Mes can be used in the medium. Gold particles can also be substituted for tungsten; however, they are more expensive and of limited availability, especially in certain sizes. When gold cannot be used and tungsten toxicity is suspected, it is beneficial to wash the cells after bombardment, to reduce particle loads, and to avoid multiple bombardments.

Currently, the best recommendations for reducing cell injury during biolistic treatment are to use the healthiest cells possible and to use the helium device (now commercially available from Bio-Rad). The helium accelerator should be configured with the flying disc, using 1000 psi or less, long target cell distances (115 mm), medium rupture membrane to flying disc distances (9 mm), gold particles, and baffles or meshes as needed.

ACKNOWLEDGMENTS

The technical assistance of Shirlee Hardy and Cathy Rose is gratefully acknowledged. We also thank William Crosby for providing plasmids pBI505 and pBI426 and the Rumsey-Loomis Machine Shop, Ithaca, NY, for construction of the prelaunch baffle. This manuscript benefited from critical reviews by Karen Kindle and Franzine Smith.

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