Detection of Reporter Gene Activity in Cell Cultures and Murine Epidermis After Helios® Gene Gun-Mediated Particle Bombardment

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Summary

The Helios gene gun-mediated DNA transfer can be used as a fast and efficient method to transfect living cells. In this study we describe the preparation of DNA-coated microcarriers and conditions used for plasmid inoculations. Expression from these plasmids was demonstrated by localization of ß-galactosidase activity in HeLa cell monolayers and murine epidermal cells and by the detection of HeLa cells positive for the green fluorescent protein (GFP).

Introduction

The application of gene transfer by particle bombardment was first described by Sanford *et al.*, (1987) and was shown to be an efficient method for transformation of many different organisms (Klein *et al.*, 1992). One of the newest technical systems used for the delivery of DNA-coated gold microcarriers by helium gas shock wave is the Helios gene gun developed by Bio-Rad (Hercules, CA, USA). With the gene gun, a certain amount of DNA-coated microcarriers is placed onto the inner surface of small PTFE tubes. After cutting the PTFE tube, up to 12 cartridges can be placed in the cartridge holder of the Helios gene gun at the same time. A high velocity helium gas stream causes a detachment of the microcarriers from the surfaces. After that, the DNA-coated gold microcarriers are fast enough to penetrate and transform living cells under *in vitro* and *in vivo* conditions. Advantages of this technology are

Any cell, tissue, or organ that can be made accessible for the gene gun can be transformed.

No vacuum is necessary during the application.

Convenient and efficient method for gene transfer.

Fast preparation of many DNA-coated cartridges at one time.

Transformation of many specimens in a short time period.

Here we present data to demonstrate a fast and easy method for transformation of living cells using the Helios gene gun apparatus and the detection of repoter gene activity after particle bombardment under *in vitro* and *in vivo* conditions.

Materials and Methods

CELL CULTURE AND ANIMALS

HeLa cells were cultured in minimal essential medium (MEM) supplemented with 10% heat-inactivated fetal calf serum, 104 U of penicillin per ml, and 10 mg of streptomycin per ml on sterile cover slips placed in six-well tissue culture plates until the cells reached approximately 80% confluence.

Inbred male BALB/c mice, 7-9 weeks of age, were used in this study.

PLASMIDS

The plasmid pCMV-ß-gal (Clontech, Palo Alto, CA) was used in experiments demonstrating the expression of ß-galactosidase gene activity under *in vitro* and *in vivo* conditions. The plasmid pCMV without the ß-galactosidase gene was applied as a negative control. Gold microcarriers, coated with the plasmid pEGFP (Clontech, Palo Alto, CA), were kindly provided by Dr. Rommerskirch, Institute of Biochemistry, Medical Center of the Friedrich Schiller University Jena and were used for the detection of green fluorescent protein (GFP) activity in Helios gene gun-transfected HeLa cell cultures.

PREPARATION OF DNA-COATED GOLD MICROCARRIERS

Fifty mg gold microcarriers (diameter: 0.6 or 1.0 µm) and 100 µl of 0.05 M spermidine were vortexed for 5 sec and sonicated for 10 sec in an ultrasonic bath to break up gold clumps. To obtain a DNA Loading Ratio (DLR) of 2, 100 µg plasmid DNA in 100 µl distilled H_2O were added. After that, 100 µl 1 M $CaCl_2$ were added dropwise by vortexing the mixture at moderate rate. After incubating the mixture for 10 min at RT, most of the gold and DNA were precipitated. After centrifugation for 15 sec at 14.000 rpm, the pellet was washed three times in 1 ml ethanol (100%, absolute water free). After the final wash, the pellet was resuspended in 200 µl ethanol containing 0.1 mg/ml polyvinylpyrrolidone (PVP). To obtain the desired Microcarrier Loading Quantity (MLQ) of 0.5, the final volume was adjusted to 6.0 ml using the ethanol/PVP solution. This suspension was then used for tube preparation which gave a yield of 80–90 cartridges containing DNA-coated gold microcarrier with a DLR of 1 µg DNA/shot.

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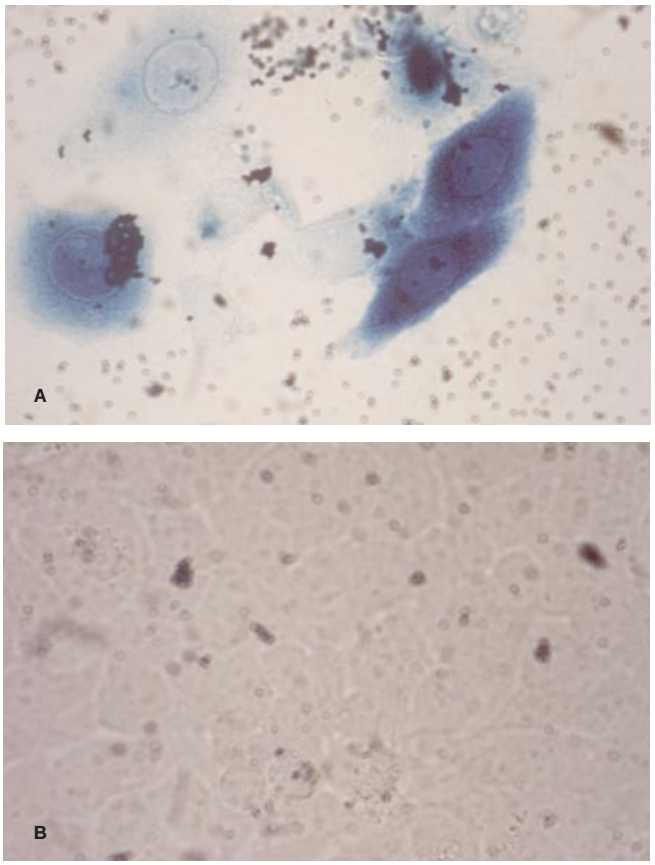

Fig. 1. Characterization of ß-galactosidase expression in pCMV-ß-gal-transfected (A) HeLa cells 48 hr after Helios gene gun-mediated DNA transfer. Several blue stained cells are present in the area of particle administration, whereas no blueness occurred in pCMV-transfected cells (B). Cells were bombarded at 50 psi with 0.6 µm gold particles and a DLR of 1 µg DNA/shot (magnification: x 500).

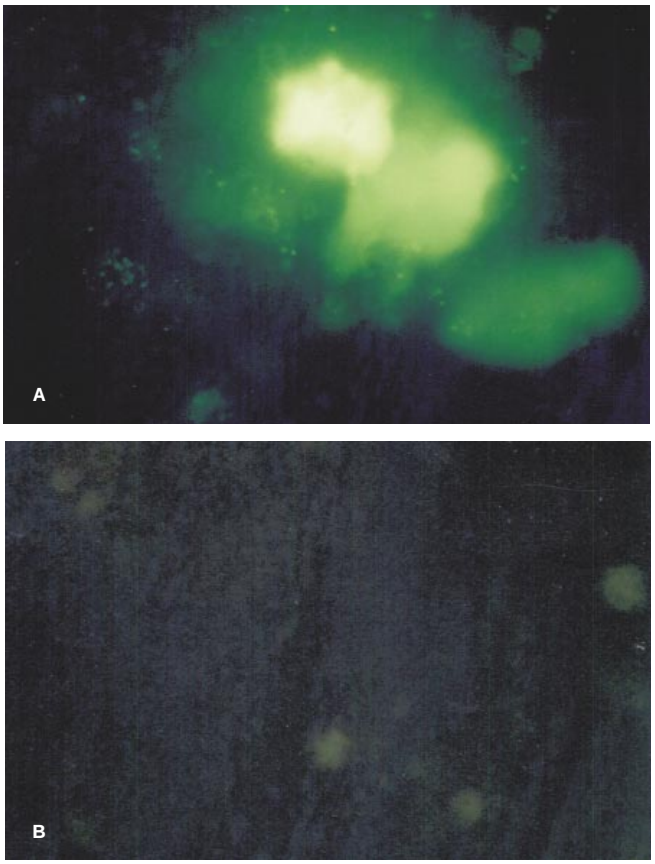

Fig. 2. Characterization of green fluorescent protein expression in pEGFP-transfected HeLa cells 48 hr after Helios gene gun-mediated DNA transfer (A). Using a LEITZ PLOEMOPAK I2/3 filter system for FITC several viable and fluorescent cells are present in the area of particle administration. No background fluorescence was detectable in non-transfected cells (B). Cells were bombarded at 50 psi with 1.0 µm gold particles (magnification: x 500).

CONDITIONS FOR PARTICLE BOMBARDMENT

***In vitro* experiments.** Immediately after aspirating the tissue culture media, confluent HeLa cell monolayers were transfected with single shots of plasmid DNA coated on 0.6 or 1.0 µm gold microcarriers. The plastic spacer of the Helios gene gun was placed as close as possible to the target and a helium pressure of 50 psi was applied. After the particle bombardment, 2 ml of media was added and the cell cultures were incubated for 48 hr at 37 ºC and 5% CO_2.

***In vivo* experiments.** One day prior particle bombardment the abdominal fur of mice was removed by shaving. Murine epidermis was transfected with 1.0 µm DNA-coated gold microcarriers by holding the plastic spacer of the Helios gene gun directly against the target site. A helium pressure of 400 psi was applied.

DETECTION OF REPORTER GENE ACTIVITY

Characterization of ß-galactosidase expression *in vitro*. Two days after particle bombardment using pCMV-ß-gal-coated 0.6 µm gold microcarriers or pCMV-coated gold microcarriers without the ß-galactosidase gene as a negative control, the efficiency of ß-galactosidase gene expression was analyzed by X-gal staining. Briefly, PBS-washed cell cultures were fixed for 10 min at 4 ºC with a fixative containing 2% paraformaldehyde and 0.2% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.3. After removing the fixative, cell cultures were washed with PBS twice, followed by adding the staining solution containing 0.1 M sodium phosphate buffer (pH 7.3), 1.3 mM $MgCl_2$, 3 mM K4Fe(CN)6, 3 mM $K_4Fe(CN)_6$, and 1 mg/ml 5-bromo-4-chloro-3-indolyl-ß-D-galactopyranoside (X-gal). The cell cultures were incubated at 37 ºC until blueness occurred, and analyzed microscopically.

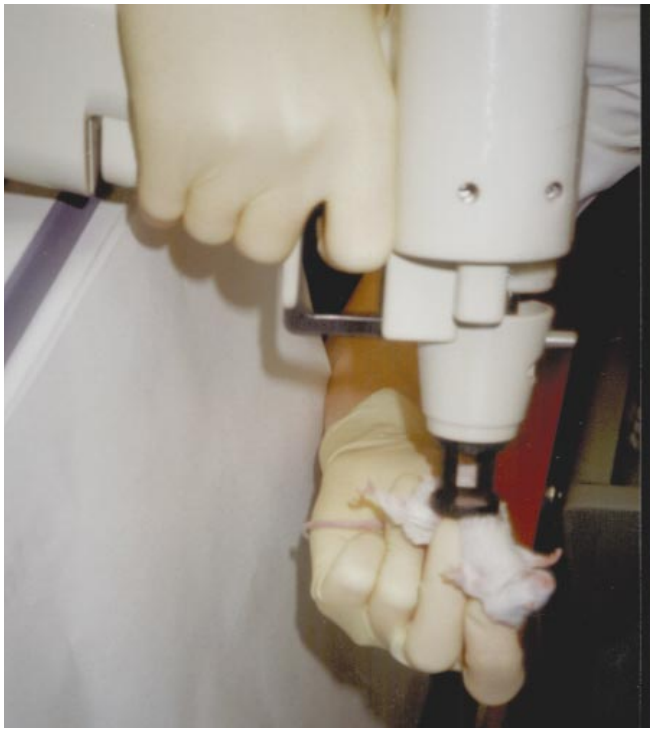

Fig. 3. In vivo *bombardment of murine epidermis using the Helios® gene gun*

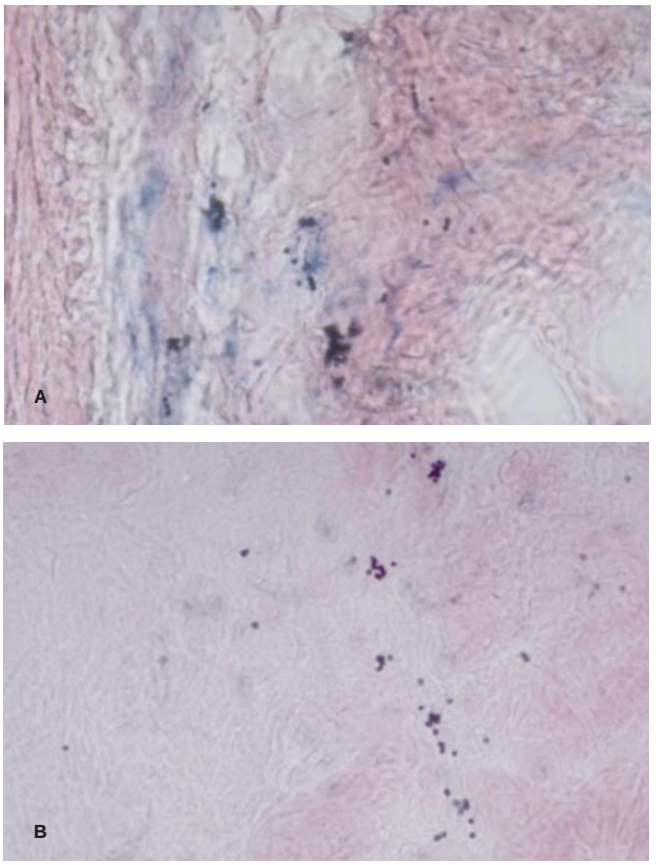

Fig. 4. Localization of ß-galactosidase expression in murine epidermal cells 7 days after bombardment with 1.0 µm pCMV-ß-gal-coated gold particles (A). Several blue stained cells are present in the area of particle administration, whereas no blueness occurred in epidermal cells of mice bombarded with 1.0 µm pCMV-coated gold particles (B). A helium pressure of 400 psi and a DLR of 1 µg DNA/shot were applied (magnification: x 500).

Characterization of ß-galactosidase expression *in vivo.* In order to analyze the applicability and efficiency of the pCMV-ß-gal expression system under *in vivo* conditions, the abdominal skin of male BALB/c mice were inoculated with pCMV-ß-gal-coated 1.0 µm gold microcarriers. Mice bombarded with the pCMV plasmid represented the negative control. One week after inoculation the skin was removed, embedded in OCT, and quick frozen using liquid nitrogen. Every fifth 10 µm air-dried section was histochemically stained for ß-galactosidase activity using the staining procedure described above.

Characterization of GFP expression *in vitro.* Two days after transfection with pEGFP-coated 1.0 µm gold microcarriers, the expression of GFP in HeLa cell cultures was analyzed using a standard fluorescence microscope.

Results and Discussion

Detection of reporter gene activity *in vitro.* HeLa cell monolayers were transfected with a single particle bombardment of 0.6 µm pCMV-ß-gal-coated and 1.0 µm pEGFP-coated microcarriers. pCMV-transfected cells were used as a negative control for ß-galactosidase expression experiments. A helium pressure of 50 psi was applied in all *in vitro* experiments decreasing gas pressure-caused cell destruction. When higher helium pressures were used destruction of cell monolayers occurred after gold particle administration. Two days after transfection pCMV-ß-gal and pCMV-transfected cells were fixed and analyzed for ß-galactosidase activity. Many ß-galactosidase positive cells were easily detectable in the area of administered gold particles, as shown in Figure 1A. In contrast, in the area particle bombardment, ß-galactosidase activity was absent in pCMV-transfected cells, as demonstrated in Figure 1B. Furthermore, bright fluorescence was present in the area of administrated gold particles shown by many viable HeLa cells transfected with the plasmid pEGFP (see Figure 2A). A background fluorescence of non-transfected cells was almost not detectable. By analyzing the reporter gene activity of both plasmids used, we were able to show that the Helios gene gun-mediated DNA transfer is a fast, convenient, and efficient method for gene transfer *in vitro* using minimal amounts of DNA.

Detection of reporter gene activity *in vivo.* Epidermal cells of male BALB/c mice were transfected with a single particle bombardment using a helium pressure of 400 psi and 1.0 µm microcarriers. The plastic spacer of the Helios gene gun was held directly against the target site as shown in Figure 3. Thereafter, no apparent skin disruption, hematoma, or bleeding were observed. One week later, mice were scarified and abdominal skin was analyzed for the presence of ß-galactosidase. Several blue stained epidermal cells were detectable in murine skin bombarded with pCMV-ß-gal-coated microcarriers as it is demonstrated in Figure 4A. No blueness occurred in murine epidermal cells bombarded with pCMV-coated microcarriers (Figure 4B) demonstrating the specificity of the expression induced by the Helios gene gun-mediated DNA transfer. Using this method we found that microcarrier-mediated gene transfer under *in vivo* conditions a fast, convenient, and efficient procedure to express foreign DNA using only a minimal amount of plasmids.

References

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2 *Sanford, J. C., Klein, T. M., Wolf, E. D. and Allen, N., Delivery of substances into cells and tissue using a particle bombardment process,* Part. Sci. Technol., **5,** *27-37 (1987).*

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