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Technical Report

Gene Gun Bombardment with DNA-Coated Gold Particles Is a Potential Alternative to Hydrodynamics-Based Transfection for Delivering Genes into Superficial Hepatocytes

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ABSTRACT

Although *in vivo* nonviral gene delivery to the liver is critical for hepatic gene therapy, there are a number of technical obstacles. Enhanced green fluorescent protein (EGFP)-encoding DNA was coated onto gold particles (gold–DNA), dissolved in phosphate-buffered saline (pure DNA), and prepared as a polymer adjuvant (jetPEI)–galactosidase solution (polymer–DNA). Murine liver transfection was attempted by nonviral approaches, which included hydrodynamics-based transfection (HBT) of pure DNA, transport and transhepatic injection of polymer–DNA, and gene gun bombardment with pure DNA, gold–DNA, and polymer–DNA. Only HBT and gene gun bombardment yielded significant numbers of $EGFP^+$ hepatocytes. With the exception of the edge of the liver, HBT had a whole-liver transfection rate of 20% under optimized conditions. HBT resulted in marked hepatic infarctions, most prominently at the edge of the liver. For gene gun bombardment, the transfection rate was pressure dependent and limited to 15% for gold–DNA. Triple or quadruple bombardment at 30 psi resulted in a transfection rate comparable to that of a single bombardment at higher pressure, but was associated with minimal scattered hepatic necrosis. The $EGFP^+$ hepatocytes were located mainly in the superficial layers. We conclude that both HBT and gene gun bombardment yielded efficient murine hepatocyte transfection *in vivo*. Severe hepatic infarction impedes foreign gene expression in the superficial hepatocytes after HBT. Repeated bombardment with gold–DNA, using an accelerated particle gene gun at 30 psi, is a potential alternative to HBT for delivering genes to superficial hepatocytes *in vivo*, although gold-related hepatic necrosis is a persistent problem.

INTRODUCTION

In vivo gene delivery to the liver is critical for both experimental and clinical applications. At present, there are two main modes for gene delivery: viral and nonviral (Dobson, 2006). Viral vectors confer more effective expression than synthetic molecular gene vectors, albeit at the expense of infection and immunogenicity (Azzam and Domb, 2004). To lessen the potential biohazards of viral vectors, naked DNA is considered attractive because it can be manipulated by standard recombinant DNA techniques and delivered by both chemical and physical means. However, chemical approaches such as circulating cationic vectors can attract serum proteins, leading to dynamic changes in their physicochemical properties and diminished transfection efficiency (Nishikawa and Huang, 2001). Physical approaches to gene transfer have improved and become as effective as viral vectors (Wells, 2004). Hydrodynamics-based transfection (HBT) of hepatocytes has been reported to produce

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a satisfactory transfection efficiency in mice (Wolff and Budker, 2005). Notably, gene guns can be used for difficult-to-transfect cells and particular *in situ* approaches (Johnston and Tang, 1994). However, whether gene guns are effective for liver transfection is uncertain. We examined the effectiveness of murine liver transfection by gene gun bombardment with enhanced green fluorescent protein (EGFP)-encoding DNA and compared the results with those obtained by other chemical or physical approaches.

MATERIALS AND METHODS

Mice

Eight-week-old male FVB/N mice were purchased from the Animal Center of the National Science Council (Taipei, Taiwan). For each transfection method, 30 mice were used. The use of animals in this study was approved by the Animal Care and Use Committee at Chang Gung Memorial Medical Center (Taoyuan, Taiwan).

Preparation of DNA

EGFP plasmid (PEGFP-C1, 4.7 kDa) was purchased from Clontech (Mountain View, CA). The plasmid was cloned and purified with an EndoFree plasmid kit (Qiagen, Valencia, CA). Naked EGFP DNA was dissolved at 1 μg/μl in phosphate-buffered saline (PBS) (pure DNA). EGFP DNA-coated gold particles (gold–DNA) were prepared by adding 5 mg of Biolistic 1.0-μm gold particles (Bio-Rad, Hercules, CA) to 5 μl of 1-μg/μl plasmid solution, 20 μl of 0.1 *M* spermidine (Sigma-Aldrich, St. Louis, MO), and 20 μl of 0.5 *M* $CaCl_2$ (Sigma-Aldrich). After several washes, the precipitate was dissolved in 100% alcohol for bombardment. The EGFP DNA–jetPEI–Gal solution (polymer–DNA) was prepared according to the manufacturer's protocol (Polyplus Transfection, New York, NY). The ratio of nitrogen residues on jetPEI to phosphates on the DNA backbone (N:P ratio) ranged from 5 to 10 for 0.31 to 0.62 μg of DNA.

Gene gun transfection with pure DNA, gold–DNA, and polymer–DNA

After general anesthesia by intraperitoneal injection of ketamine and diphenhydramine (Benadryl; Pfizer, New York, NY), the mice underwent midline laparotomy, to exposure the liver for gene gun bombardment. *In situ* liver transfections were performed with the low pressure-accelerated particle gene gun (Bioware Technologies, Taipei, Taiwan). A 1-cm-thick rubber ring was placed on the shooting end of gene gun. Briefly, gold–DNA (5–20 μl) was bombarded into mouse liver at pressures of 20–45 psi. Alternatively, pure DNA (5–20 μl) was bombarded into mouse liver at pressures of 20–45 psi. For polymer–DNA bombardment, DNA–jetPEI–Gal solution (5 μl) was bombarded into mouse liver. The mouse abdomen was closed carefully after bombardment.

Intravenous or direct liver injection of polymer–DNA

Mouse liver was exposed as described above. EGFP DNA–jetPEI–Gal solution (N:P ratio, 5–10) was used for *in vivo* transfection via the portal vein (100 to 400 μl for 10 min) or direct injected into the right lobe of the liver (20 to 100 μl for 3 min). Tail vein injection was also performed (400 μl for 10 min) without laparotomy.

Hydrodynamics-based transfection

Five to 250 μg of EGFP DNA was injected via the tail vein in a volume of saline equivalent to 8% of the body mass of the mouse (e.g., 1.6 ml for a 20-g mouse). The entire volume was delivered within 5 sec.

Transfection rate evaluation

Mice were killed 48 hr or 7 days after transfection, and their livers were harvested. The livers were either cryofixed or fixed in 4% buffered paraformaldehyde (PFA). Unless otherwise indicated, transfection rates were evaluated 48 hr after transfection.

Cryofixation was performed by immersion of tissues in ice-cold isopentane for 3 min, followed by freezing at -80°C. Fixed frozen samples were mounted in Tissue-Tek O.C.T. 4583 compound (Sakura Finetek USA, Torrance, CA). Samples were sectioned sequentially on a Jung Frigocut 2800N (Leica, Deerfield, IL) at a cutting interval of 6 μm. Samples fixed in 4% PFA were subjected to hematoxylin and eosin (H&E) staining. Sections were examined by either fluorescence microscopy or light microscopy. $EGFP^+$ hepatocytes were observed at $\times$20 magnification under the fluorescence microscope. The transfection rate was defined as the number of $EGFP^+$ hepatocytes divided by the total number of hepatocytes within the same field on three randomized occasions. Mice transfected with DNA-free PBS (with or without gold) of the same volume were used as negative controls.

Hepatic inflammation evaluation

Forty-eight hours after transfection, the serum alanine aminotransferase (ALT) levels of the mice were measured with a Vitros DT60 II chemistry system (Johnson & Johnson, New Brunswick, NJ).

Statistical analysis

Independent sample *t* testing was used to compare the means obtained for two different bombardment pressures or repetitions. One-way analysis of variance (ANOVA) was used to test the equality of the means among the three DNA groups. Differences were regarded as significant for $p < 0.05$.

RESULTS

Gene gun transfection with pure DNA, gold–DNA, and polymer–DNA

Mice transfected with the EGFP plasmid by gene gun bombardment did not have significant numbers of $EGFP^+$ hepatocytes unless a pressure of 30 psi was used (Figs. 1A and 2). With respect to transfection rate, gold–DNA compared favorably with pure DNA and polymer–DNA (Fig. 2). However, liver laceration increased abruptly at pressures above 30 psi, ac-

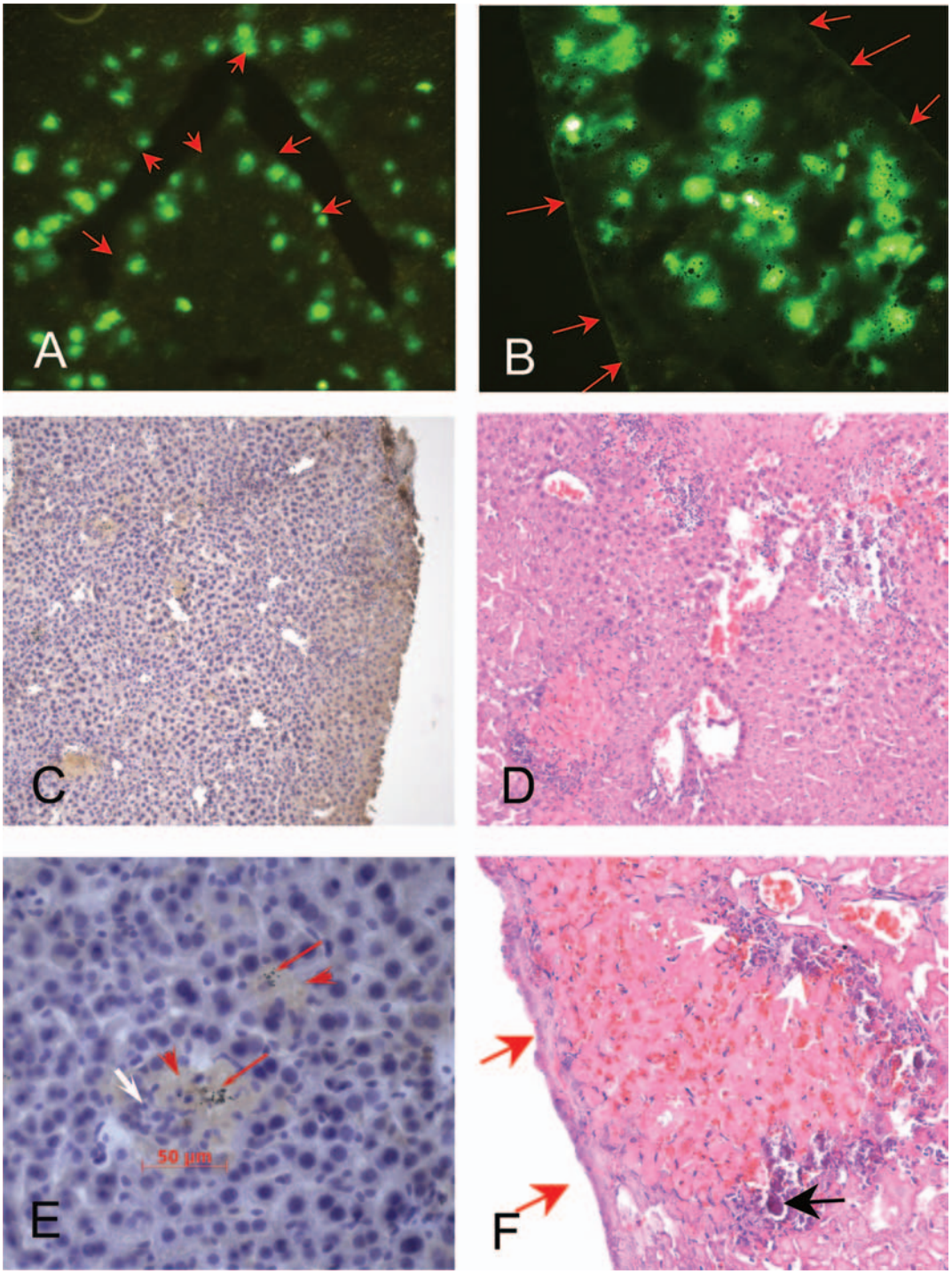

FIG. 1. (**A** and **B**) EGFP$^+$ hepatocytes are shown (original magnification, ×20) after gene gun bombardment with gold–DNA (**A**) at a pressure of 30 psi, and after HBT with injection of 10 μg of DNA within 4 sec (**B**). The edge of the liver is indicated by red arrows. (**C** and **E**) H&E staining of mouse liver after gold–DNA bombardment; a low-power field (**C**, ×100) and a high-power field (**E**, ×400) are shown. Gold particles (red arrows) and inflammatory cells (white arrow) are scattered in an area of necrotic hepatocytes (red arrowheads). (**D** and **F**) H&E staining of mouse liver after HBT; a low-power field (**D**, ×100) and a high-power field (**F**, ×400) are shown. Diffuse infarctions in the hepatic parenchyma are evident (**D**). A representative confluent hepatic infarction (**F**, red arrows) is located underneath the edge of the liver. Infiltrating inflammatory cells (**F**, white arrows) and calcification (**F**, black arrow) are associated with the infarction.

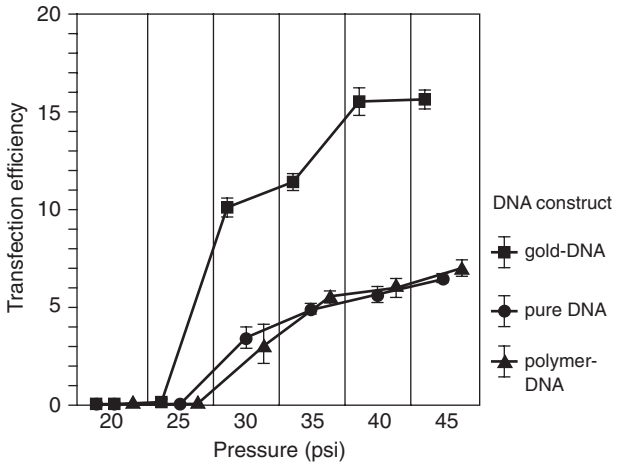

FIG. 2. Relationships between the transfection rate and bombardment pressure for three DNA preparations. In terms of transfection efficiency, gold–DNA compares favorably with pure DNA and polymer–DNA at a pressure of :30 psi ($p <$ 0.001, one-way ANOVA). For each DNA preparation, significant differences were observed for 25 versus 30 psi, 30 versus 35 psi, 35 versus 40 psi, and 40 versus 45 psi ($p < 0.001$–0.044, *t* test), but not for 40 versus 45 psi in the gold–DNA group ($p = 0.754$, *t* test).

counting for a mortality rate of more than 35%. The maximal transfection rate achieved by a single bombardment was approximately 15% for gold–DNA and 5–6% for pure DNA or polymer–DNA (Fig. 2). At 30 psi, the transfection rates reached a plateau at approximately 6.2, 5.9, and 15% for pure DNA, polymer–DNA, and gold–DNA, respectively, with three or four bombardments (Fig. 3). The mortality rate after triple bombardment at 30 psi was negligible and ranged from 0 to 3.3%. Further repetitions of bombardment led to mortality due to gross liver laceration. Regardless of the composition of the DNA solution, $EGFP^+$ hepatocytes after bombardment were located mainly in the superficial layers (depth of 10–60 μm, one to three cell layers) of the liver. Despite the better transfection rate obtained for gold–DNA, H&E staining of bombarded liver tissues revealed several necrotic spots with deposition of gold particles (Fig. 1C and E), indicating liver injury at the bombardment site, probably caused by the gold particles. The ALT levels of the mice were 249 ± 75 U/liter (normal range, 15–84 U/liter). One week after bombardment with gold–DNA, the transfection rate decreased to 9.7%.

Intravenous and direct liver injection of polymer–DNA

None of the transfections with polymer–DNA generated $EGFP^+$ hepatocytes.

Hydrodynamics-based transfection

The immediate mortality rate was 6.6%, despite cardiopulmonary resuscitation for more than 10 min. The highest transfection rate for HBT was about 20% under optimized conditions of :10 μg of DNA injected within 4 sec. $EGFP^+$ hepatocytes were evenly distributed over the whole liver but were scarce at the edge of the liver (Fig. 1B, arrows). H&E staining revealed remarkable hepatic infarctions in both control and experimental animals. At the edge of the liver, confluent infarctions were impressive and formed broad bands (Fig. 1D and F). The ALT levels of mice were 588 ± 135 U/liter. One week after HBT, the transfection rate decreased to 11.8%.

DISCUSSION

The chemical approach with jetPEI-Gal injection in FVB/N mice was unsatisfactory, as it gave minimal transfection rates regardless of the injection route. Successful *in vivo* transfections by jetPEI injection have been reported in the lung (Zou *et al*., 2000). Although jetPEI-Gal was chosen over jetPEI for use in the current study, because of its higher affinity for hepatocytes (Robaczewska *et al*., 2001), our data indicate that the liver represents a more robust barrier for polymer–adjuvant transfection than the lung.

HBT yielded the highest transfection rate of all the nonviral DNA delivery methods. This is comparable to the results of previous studies (Zhang *et al*., 1999; Yang *et al*., 2002). However, rapid injection of a large volume via the tail vein usually causes transient heart dysfunction (Zhang *et al*., 2004) and may lead to animal loss. Clinical application is not feasible, because humans lack a homolog for the tail vein. Furthermore, HBT leads to increased venous pressure (Zhang *et al*., 2004) and subsequent hepatic infarction. The infarctions had a tendency to be confluent at the liver edge, where perfusion is sparser than elsewhere. Thus, it does not guarantee foreign gene expression in the superficial hepatocytes.

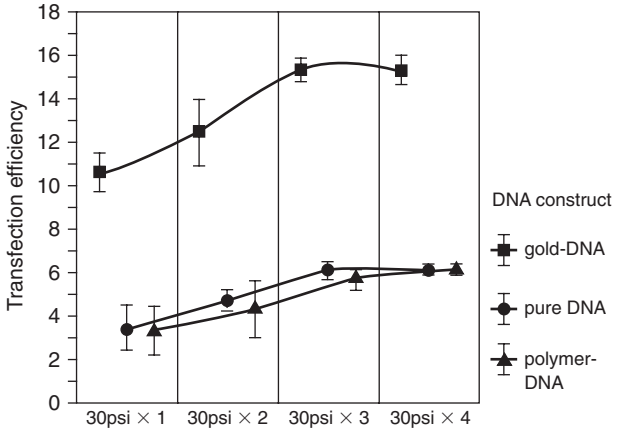

FIG. 3. Transfection rates in relation to number of bombardment repetitions at a pressure of 30 psi. For the same number of bombardment repetitions, gold–DNA gives superior transfection rates compared with pure DNA and polymer–DNA ($p <$ 0.001, one-way ANOVA). A significant increase in transfection rate is observed for bombardment performed up to three times for each DNA group ($p < 0.001$–0.032, *t* test), with the exception of polymer–DNA bombardment carried out once and twice ($p = 0.075$, *t* test). The transfection rates for three and four bombardment repetitions are not significantly different for each group ($p = 0.55$–1.0, *t* test).

The original application of the gene gun was for skin vaccination, which induces DNA expression in the most superficial layers of the skin (Johnston and Tang, 1994; Peachman *et al*., 2003). Thus, cell sampling for gene gun bombardment should focus on the superficial cells. GFP^+ hepatocytes were most prominent in the first three layers. In comparison with the skin, the liver is too fragile to bear the bombardment pressure required for *in situ* transfection. Therefore, the pressure must be adjusted by weighing transfection efficiency against possible liver tearing. Triple bombardment at a tolerable pressure of 30 psi has been shown to yield a transfection efficiency comparable to that obtained from a single bombardment at higher pressure, which usually causes gross liver laceration. However, unpredictable location of gene transfer usually ensues from a direct strike (our unpublished data). Therefore, a rubber ring was placed at the opening of the gene gun. It ensures good guidance, allowing constant focusing and an attenuated blast effect. Among the various DNA preparations, gold–DNA bombardment had the highest transfection rate, although it was associated with gold particle-related necrosis. Nevertheless, the level of injury, determined by histological examination and the serum ALT level, was less severe than that caused by HBT. Gene gun bombardment is comparable with HBT in terms of stability, with a 20% decrease in the transfection rate after 1 week.

In conclusion, gene gun bombardment of the liver with gold–DNA is a potentially useful alternative to HBT for the transfection of superficial hepatocytes, particularly because it does not induce severe hepatic injury. Its application could potentially be extended to other animals, regardless of the presence or absence of a tail vein; however, its application is limited to superficial cells and animals that can tolerate laparotomy.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

AZZAM, T., and DOMB, A.J. (2004). Current developments in gene transfection agents. Curr. Drug Deliv. **1,** 165–193.

DOBSON, J. (2006). Gene therapy progress and prospects: Magnetic nanoparticle-based gene delivery. Gene Ther. **13,** 283–287.

JOHNSTON, S.A., and TANG, D.C. (1994). Gene gun transfection of animal cells and genetic immunization. Methods Cell Biol. **43,** 353–365.

NISHIKAWA, M., and HUANG, L. (2001). Nonviral vectors in the new millennium: Delivery barriers in gene transfer. Hum. Gene Ther. **12,** 861–870.

PEACHMAN, K.K., RAO, M., and ALVING, C.R. (2003). Immunization with DNA through the skin. Methods **31,** 232–242.

ROBACZEWSKA, M., GUERRET, S., REMY, J.S., CHEMIN, I., OFFENSPERGER, W.B., CHEVALLIER, M., BEHR, J.P., PODHAJSKA, A.J., BLUM, H.E., TREPO, C., and COVA, L. (2001). Inhibition of hepadnaviral replication by polyethylenimine-based intravenous delivery of antisense phosphodiester oligodeoxynucleotides to the liver. Gene Ther. **8,** 874–881.

WELLS, D.J. (2004). Gene therapy progress and prospects: electroporation and other physical methods. Gene Ther. **11,** 1363–1366.

WOLFF, J.A., and BUDKER, V. (2005). The mechanism of naked DNA uptake and expression. Adv. Genet. **54,** 3–20.

YANG, P.L., ALTHAGE, A., CHUNG, J., and CHISARI, F.V. (2002). Hydrodynamic injection of viral DNA: A mouse model of acute hepatitis B virus infection. Proc. Natl. Acad. Sci. U.S.A. **99,** 13825–13830.

ZHANG, G., BUDKER, V., and WOLFF, J.A. (1999). High levels of foreign gene expression in hepatocytes after tail vein injections of naked plasmid DNA. Hum. Gene Ther. **10,** 1735–1737.

ZHANG, G., GAO, X., SONG, Y.K., VOLLMER, R., STOLZ, D.B., GASIOROWSKI, J.Z., DEAN, D.A., and LIU, D. (2004). Hydroporation as the mechanism of hydrodynamic delivery. Gene Ther. **11,** 675–682.

ZOU, S.M., ERBACHER, P., REMY, J.S., and BEHR, J.P. (2000). Systemic linear polyethylenimine (L-PEI)-mediated gene delivery in the mouse. J. Gene Med. **2,** 128–134.

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