THE JOURNAL OF GENE MEDICINE
J Gene Med 2006; **8**: 353–361.
Published online 14 December 2005 in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/jgm.851

RESEARCH ARTICLE

Mechanism of *in vivo* DNA transport into cells by electroporation: electrophoresis across the plasma membrane may not be involved

Feng Liu[1]*
Steve Heston[2]
Lisa M Shollenberger[3]
Bin Sun[3]
Marlin Mickle[2]
Michael Lovell[2]
Leaf Huang[1]

[1]*School of Pharmacy, University of North Carolina, Chapel Hill, NC, USA*
[2]*School of Engineering, University of Carolina, Chapel Hill, NC, USA*
[3]*School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA*

*Correspondence to: Feng Liu, University of North Carolina School of Pharmacy, Chapel Hill, NC 27599, USA. E-mail: fliu@email.unc.edu

Received: 4 July 2005
Accepted: 20 September 2005

Abstract

Background Recently, *in vivo* gene transfer with electroporation (electro-gene transfer) has emerged as a leading technology for developing nonviral gene therapies and nucleic acid vaccines. The widely hypothesized mechanism is that electroporation induces structural defects in the membrane and provides an electrophoretic force to facilitate DNA crossing the permeabilized membrane. In this study, we have designed a device and experiments to test the hypothesis.

Methods In this study, we have designed a device that alternates the polarity of the applied electric field to elucidate the mechanism of *in vivo* electro-gene transfer. We also designed experiments to challenge the theory that the low-voltage (LV) pulses cannot permeabilize the membrane and are only involved in DNA electrophoresis, and answer the arguments that (1) the reversed polarity pulses can cause opposing sides of the cell membrane to become permeabilized and provide the electrophoresis for DNA entry; or (2) once DNA enters cytoplasmic/endosomal compartments after electroporation, it may bind to cellular entities and might not be reversibly extracted. Thus a gradual buildup of the DNA in the cell still seems quite possible even under the condition of the rapid reversal of polarity.

Results Our results indicate that electrophoresis does not play an important role in *in vivo* electro-gene transfer.

Conclusions This study provides new insights into the mechanism of electro-gene transfer, and may allow the definition of newer and more efficient conditions for *in vivo* electroporation. Copyright © 2005 John Wiley & Sons, Ltd.

Keywords electroporation; DNA transport; mechanism; muscle; gene therapy

Introduction

Naked plasmid DNA (pDNA) has been considered the simplest and safest gene delivery system. It has been known for more than a decade that naked DNA can be transferred into cells *in vivo* and result in gene expression. Mouse muscle injected with naked pDNA resulted in long-term protein expression [1]. Plasmid DNA was neither replicated nor integrated into the host cell genome, but remained in its episomal form [2] and was expressed in both dividing and nondividing cells. However, a big drawback of naked plasmid DNA as a vector for gene therapy is its low efficiency of gene expression.

Copyright © 2005 John Wiley & Sons, Ltd.

As a result, naked DNA gene transfer has been mainly used for genetic immunization studies. In addition, there is high inter-individual variability in the levels of the transferred gene expression. The reason for the lack of reproducibility is not known, but it may present a serious problem in clinical settings [3].

Recently, gene delivery *in vivo* using electroporation has become an area of greater research interest [4]. The transfection efficiency by electroporation is many times greater than that of naked DNA injection, with markedly reduced inter-individual variability [5,6]. Gene transfer by electroporation *in vivo* has been effective for introducing DNA into rat hepatocellular carcinomas, hepatocytes, mouse testes, melanoma, skeletal muscle, skin, lung and rat skeletal muscle for correcting anemia of renal failure [7–13]. Particularly, skeletal muscle has been shown to be the most promising tissue for electro-gene transfer in several contexts: (i) There is no significant cell replacement in skeletal muscle tissue, so that introduced genes are not rapidly lost following mitosis [14]; transgene expression can persist for relatively long periods [15]; and (ii) Skeletal muscle has an abundant blood vascular supply, thus providing an efficient transport system for the carriage of secreted protein into the circulation. Therefore, applications of electroporation have been extended to using muscle for systemic delivery of therapeutic proteins [16,17].

Electroporation as a gene delivery strategy combines naked DNA injection with pulsed electric-field treatment. The principles of electroporation are based on the assumption that the cell membrane, normally not permeable to charged macromolecules, is physically equivalent to an electrical capacitor [18,19]. The lipidic membrane can be considered as a dielectric element placed between the extracellular environment and the cytoplasm. When cells are exposed to an electric field, structural defects in the membrane or opening and enlarging pores will be induced, which permits the transit diffusion of charged macromolecules such as nucleic acids [18,19]. The general mechanism of electro-gene transfer clearly begins with a temporary increase in membrane permeability resulting from the electric pulses, followed by the diffusion of molecules through the membrane [17,19,20]. The question is how DNA diffuses across the permeabilized membrane, through a passive mechanism or through the effects of electrophoretic force. It has been suggested that the increased expression rates for *in vivo* DNA electro-transfer in muscle tissue are due to the enhanced movement of DNA by electrophoresis, following membrane permeabilization [21–23]. Several publications point to this model of *in vivo* DNA electro-transfer [3,21–23]. However, the true mechanism is not yet fully understood.

In this study, we have designed a device that alternates the polarity of the applied electric field to elucidate the mechanism of *in vivo* electro-gene transfer. If there is DNA electrophoresis driving the DNA into the cells, DNA will be forced into the cells at the anode during the first pulse. When the polarity is reversed during the second pulse, the DNA will be drawn back to the opposite side (cathode), and so forth. As a result, the DNA cannot move into the cells in such a way as to diminish gene expression. We also designed an experiment to answer the arguments that (1) the reversed polarity pulses can cause opposing sides of the cell membrane to become permeabilized and provide the electrophoresis for DNA entry; or (2) once DNA enters cytoplasmic/endosomal compartments after electroporation, it may bind to cellular entities and might not be reversibly extracted. Thus a gradual buildup of the DNA in the cell still seems quite possible even under the condition of the rapid reversal of polarity. Our results indicate that electrophoresis does not play an important role in *in vivo* electro-gene transfer. This study provides new insights into the mechanism of electro-gene transfer, and may allow the definition of newer and more efficient conditions for *in vivo* electroporation, such as an increased diffusion rate for DNA.

Materials and methods

Materials

PNGVL3-Luc containing the cDNA of firefly luciferase driven by the cytomegalovirus (CMV) promoter was custom prepared by Bayou Biolabs (Harahan, LA, USA) and was the kind gift of the National Gene Vector Laboratory. 2,2,2-Tribromoethanol and Evans Blue dye were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA). A BTX T820 square-wave electroporater was purchased from Genetronics (San Diego, CA, USA). Animals were kept at the University of Pittsburgh Central Animal Facility. All experiments were conducted under protocols approved by the Institutional Animal Care and Use Committee.

Electro-transfer of DNA

Mice (three per group) were anesthetized with an intraperitoneal injection of 8 mg of 2,2,2-tribromoethanol and the quadriceps muscles were surgically exposed. The syringe electrode, which was developed by our laboratory [7], was positioned so that one needle was inserted into the quadriceps parallel to the long axis of the muscle fiber and the other needle was inserted into the gastrocnemius. A DNA solution was injected into the quadriceps muscle through one syringe of the electrode immediately prior to application of current. The polarity applied in the quadriceps can be either anodic or cathodic. Unless otherwise stated, 10 µg of DNA in 10 µl of 0.9% saline was injected into the quadriceps. When using caliper electrodes, electric pulses were immediately applied to the muscle by caliper electrodes with 1-cm^2 plates (Genetronics). Electric-field strength is reported in terms of the ratio of the applied voltage to the distance between the electrodes (V/cm).

Copyright © 2005 John Wiley & Sons, Ltd.

Protein expression assays

Mice were sacrificed 3 days after electroporation and the quadriceps muscle was removed. Then, 1 ml of lysis buffer (0.1% Triton X-100, 2 mM EDTA, and 0.1 M Tris-HCl pH 7.8) was added and the muscle was homogenized using a tissue tearor. After centrifugation at 14 000 rpm for 2 min, a 10-µl aliquot of the supernatant was analyzed for luciferase activity. Luminescence was measured for 10 s in each assay, and the luciferase activity for the each assay is presented as relative light units (RLU) per quadriceps [Luciferase protein (pg) $= 7.98 \times 10^{-5}$RLU + 0.093RLU, $R^2 = 0.99$].

Results

Design of the device to deliver pulses with alternating polarity

The custom-made electronic device, as shown in Figure 1A, consists of a pulse generator, a switching circuit and data-acquisition equipment. In order to generate the alternating polarity voltage signal, the square wave created by the pulse generator (T820) was passed through the switching circuit. In this way, the direction of electrical potential across the electrodes was reversed for consecutive peaks. The data, the applied voltage signal and the resistive response of the tissue were recorded with the data-acquisition equipment. As shown in Figures 1B and 1C, this device successfully delivered electrical pulses with either consistent (nonalternating) polarity or alternating polarity.

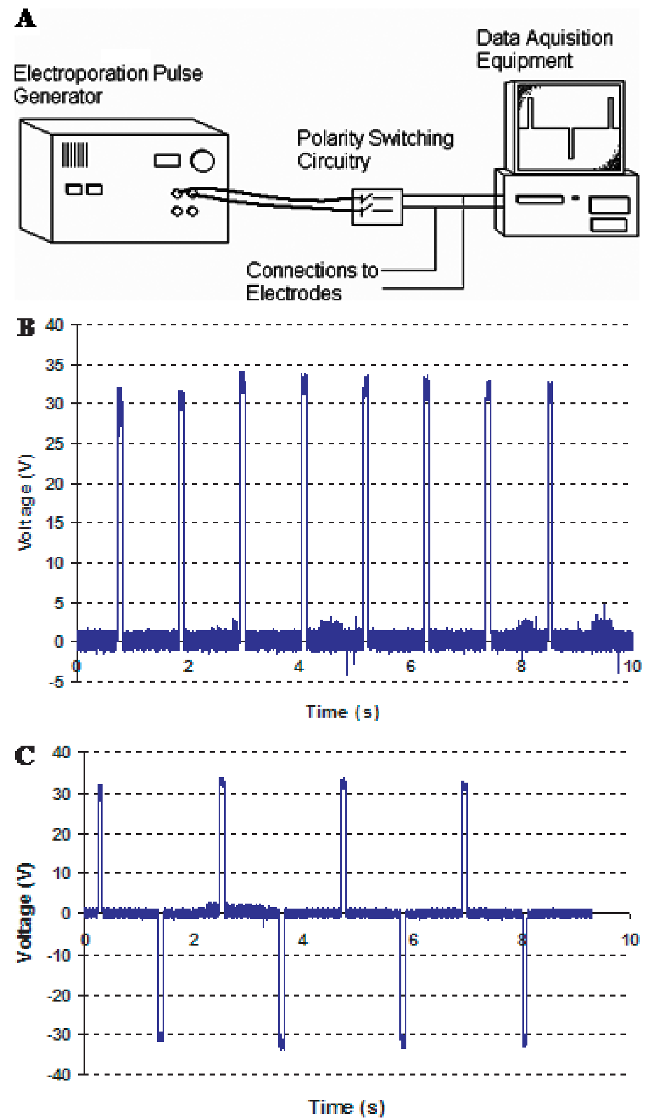

Figure 1. Pulse delivery equipment that we designed and built to generate pulses with consistent and alternating polarity. (A) Pulse signal generated by our custom-made device with (B) consistent polarity and (C) alternating polarity

Electro-gene transfer into muscle with consistent or alternating polarity

Luciferase plasmid (10 µg) was injected into mouse quadriceps, and electric pulses were delivered through either caliper or syringe electrodes. Two types of pulses, short-duration (20 µs) and long-duration (20 ms), were used in this experiment. Comparison of the results, as shown in Figure 2A, revealed that there was no significant difference in the levels of luciferase expression under pulses with either the consistent or alternating polarity ($p > 0.1$). If the electrophoretic force is a contributing mechanism in electro-DNA transfer, an alternating polarity signal should produce lower gene expression rates than those of a consistent polarity signal. To test this possibility, the effect of the pulse duration on gene expression was examined. The pulses were delivered by the caliper electrodes and the pulse duration was varied from 2 to 20 ms. The data in Figure 2B indicated that the efficiency of gene transfer increased with an increase in the pulse duration (200 V/cm, 6 pulses). However, no significant difference in gene expression was observed when the pulses were delivered either with the consistent or with the alternating polarity. The same pattern, as shown in Figure 2C, was also obtained when increasing the number of pulses (200 V/cm, 20 ms). These results again indicate that electrophoresis was not involved in the enhancement of electro-gene transfer.

Gene transfer with a combination of HV and LV pulses and LV pulses alone

A technique combining two pulses, high-voltage (HV) and low-voltage (LV), was developed for *in vivo* gene transfer by Bureau and colleagues [23]. They proposed that there are two active components in the delivered electric pulses: cell electropermeabilization induced by the HV pulses and electrophoretic forces on DNA induced by the LV pulses. Similar studies by Mir's group observed that one HV pulse followed by four LV pulses resulted in 10-fold higher

Copyright © 2005 John Wiley & Sons, Ltd.

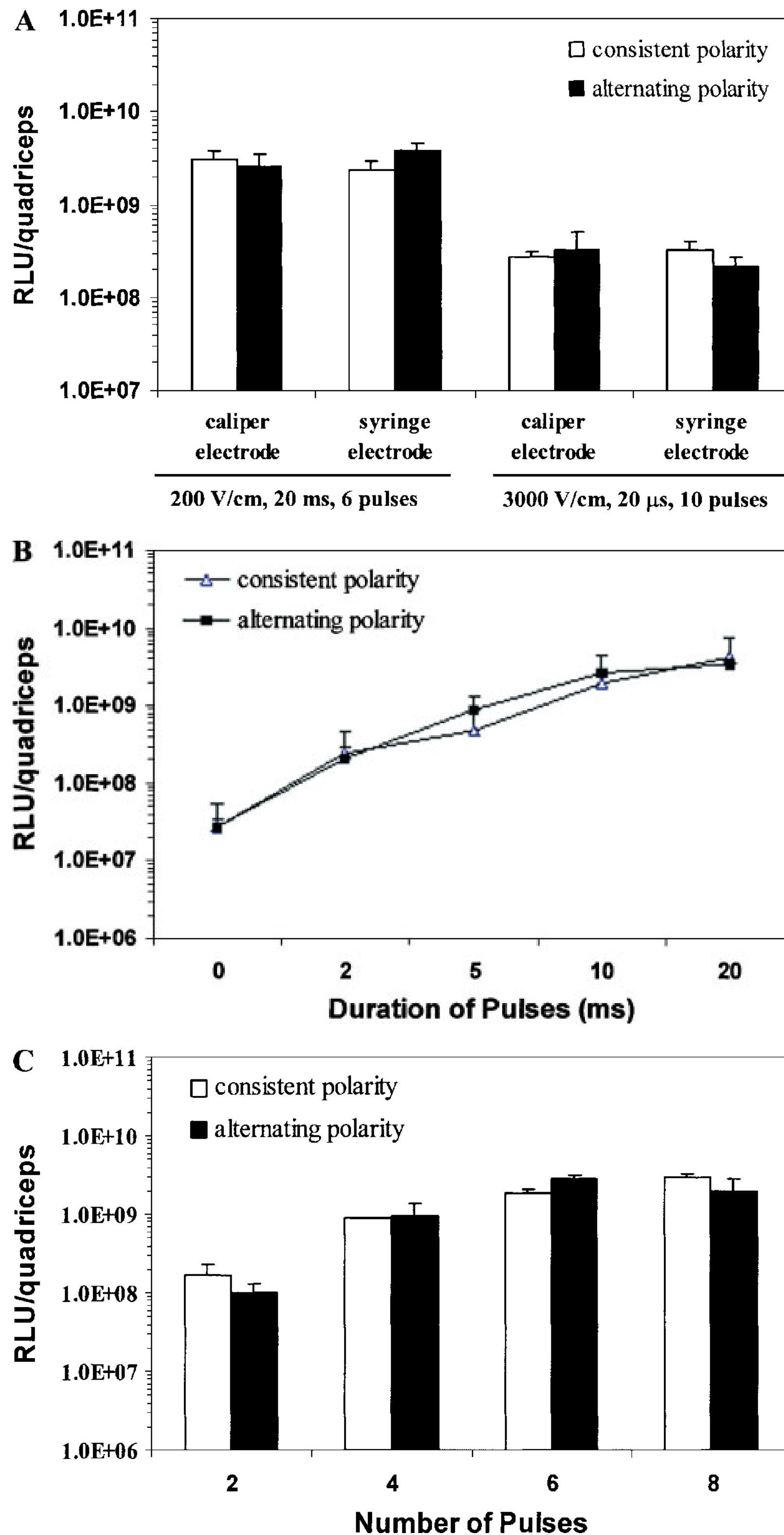

Figure 2. Luciferase expression after DNA electro-transfer into the quadriceps with the pulses of consistent or alternating polarity. (A) Electro-gene transfer was done with either caliper or syringe electrodes delivering either short (20 μs) or long (20 ms) pulses. (B, C) Luciferase expression as a function of the pulse duration and pulse number. Data represent the mean ± standard deviation (SD) (n = 3)

gene expression than one HV pulse followed by one LV pulse when the lag time between the HV and LV pulses was set from 1 to 300 s. They suggested, based on the above observations, that LV pulses play an important electrophoretic role in DNA electro-transfer. The 300 s of lag time was long enough to allow us to manually switch the pulses from HV to LV in our device so that we could replicate the studies performed by Mir's groups using

Copyright © 2005 John Wiley & Sons, Ltd.

pulses of alternating polarity. We obtained, as shown in Figure 3A, results similar to Mir's group, in which no enhancement of gene expression was detected when DNA was injected after LV pulses, but a gene could be efficiently transferred into muscle when DNA was injected either before or after delivery of HV pulses. This result also indicated that our device is reliable to perform the experiment by Mir's group. However, we did not observe any difference in efficiency of gene transfer when the LV pulses were delivered either with consistent or with alternating polarity.

It was proposed that HV pulses permeabilize the membrane, whereas the LV pulses can not permeabilize the membrane and are only involved in DNA electrophoresis [21,23]. To examine the electrophoretic properties without interference from membrane permeabilization effects, we next used six LV pulses (80 V/cm, 99 ms) to evaluate transfection efficiency. The data again showed similar levels of luciferase expression (Figure 3B) and distribution of green fluorescent protein (GFP) (Figure 3C) using either the consistent or alternating pulses in both mice and rat. Bureau *et al*. also reported that the LV pulses (4×80 V/cm) were able to promote gene transfer without inducing apparent permeabilization, as evidenced by 51 Cr-EDTA uptake [23]. However, the enhancement of gene expression as shown in Figures 3B and 3C indicated that LV pulses can induce permeabilization. To confirm that, we injected mice with Evans Blue dye (EBD) followed by six LV pulses (80 V/cm, 99 ms). The tetrasodium diazo salt Evans Blue is a membrane-impermeable molecule. This *in vivo* tracer technique provides information about certain structural and dynamic features of normal and

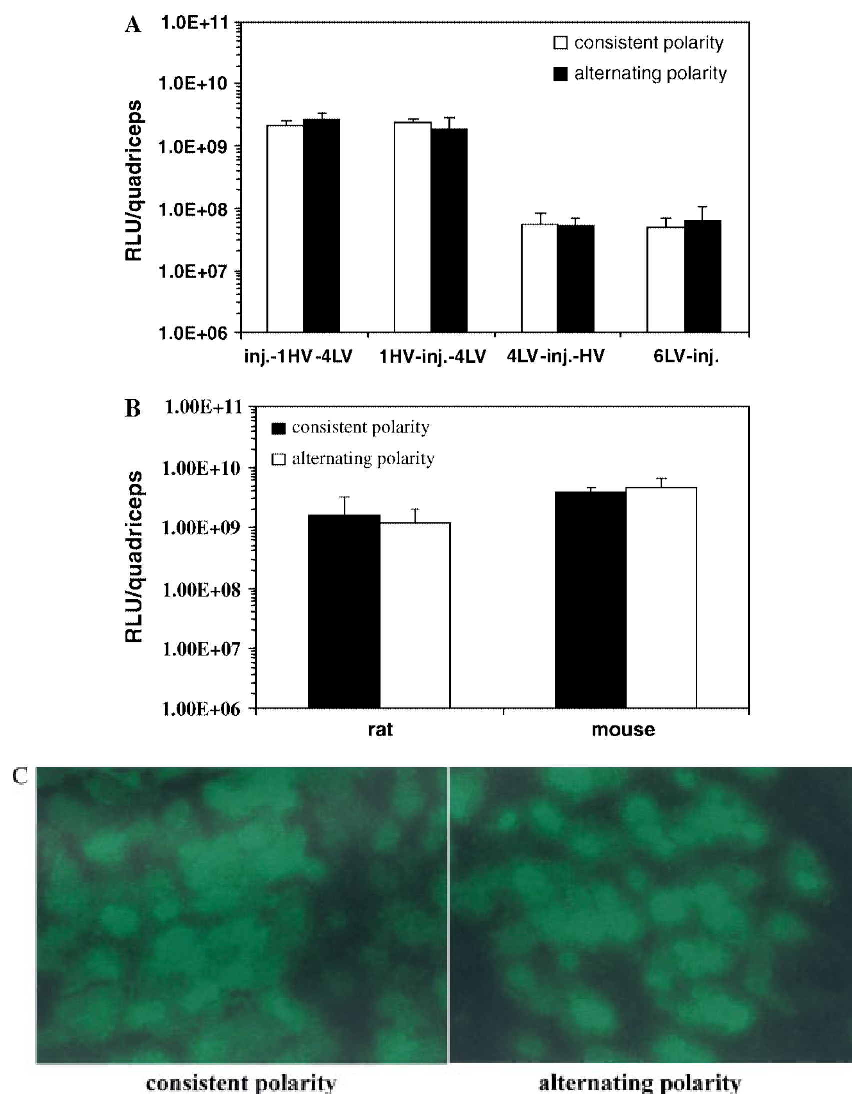

Figure 3. Luciferase expression as a function of the pulse polarity using consistent or alternating polarity. The electroporation was delivered, using the caliper electrodes, with the combination of one HV pulse (800 V/cm, 99 μs) and four LV pulses (80 V/cm, 99 ms) (A) and six LV pulses (A, B, C). DNA was injected (inj.) before or after the delivery of the HV or LV pulse (A), and the lag time between the HV and LV pulses was 30 sec. Fifty microgram luciferase DNA (B) in 100 μl of 0.9% saline and 20 μg of EGFP plasmid (pCMV-EGFP-NI) (C) were injected into the quadriceps of rat (SD, female 10 week-old rat) (B) and mice (C) respectively, followed by delivery of 6 LV pulses. For GFP assay, muscle sections (10 μm), 3 days after the injection, were mounted with Vectashield mounting medium. All sections were examined and photographed with a Nikon fluorescence microscope (×200). Data represent the mean ± SD(n = 3)

Copyright © 2005 John Wiley & Sons, Ltd.

pathological skeletal myocytes [24]. Membrane permeabilization, or tissue damage, caused by electroporation can be diagnosed by visualizing the color of the treated muscle as injected EBD selectively accumulates in the permeabilized or damaged myocytes [7]. As shown in Figure 4, myocyte uptake of EBD was detected 1 day after dye injection with a caliper electrode. Accumulated EBD was observed in the quadriceps of mice which were given the LV pulses with consistent and alternating polarity or given six pulses (200 V/cm, 200 ms), but not for the muscles without electroporation. These results again suggested that LV pulses can induce membrane permeabilization.

Other experiments showing no involvement of the electrophoretic force

The first study supporting the hypothesis of the involvement of the electrophoretic force in electro gene transfer was reported by Klenchin and colleagues [25]. They found that efficiency of gene transfer *in vitro* significantly decreased when the electrophoretic mobility of DNA is diminished by an increase in medium viscosity (from 2.5 to 10% Ficoll), as well as by adding Mg^{2+} to the medium [25]. The properties of *in vitro* electrophoresis have already been established: therefore, these results are not difficult to understand based on the fact that the electrophoretic mobility inversely depends on the viscosity of the medium, and that binding Mg^{2+} to DNA molecules can dramatically decrease the effective charge of the DNA polyanions and raise the ionic strength (increase shielding) [25]. The data in Figure 5A showed that, when DNA in a solution containing either Mg^{2+} or 10% Ficoll was injected into the muscle without electroporation, luciferase gene expression was 5 to 10 times lower than that of DNA in saline solution. However, the data in Figure 5B indicated that there was a more than 10-fold increase in gene expression with electro-gene transfer and, in particular, the levels of luciferase expression were not affected by an increase in either concentration of Ficoll or Mg^{2+}. Again, electroporation with either consistent or alternating polarity pulses made no difference in efficiency of gene transfer.

It has been reported that the plasmid interaction with the *in vitro* electropermeabilized cell surface was not affected by pulses of reversed polarities [26] because the plasmid DNA forms a complex with the cell membrane from which plasmid DNA enters the cell after the application of electrical pulses has ended [27]. These membrane complexes are not disrupted by reversing the polarity of the electrical field [28]. When changing both the pulse polarity and their direction, DNA interacts with the whole membrane cell surface [27,28] and leads to an increase in gene expression [29]. All the above observations raise the concerns: (1) the reversed polarity pulses can cause opposing sides of the cell membrane to be permeabilized, which leads to an increase in the number of muscle fibers transfected as some fibers may not be accessible to the DNA (if it is only driven by the electrophoretic force towards the membrane in one direction); or (2) once

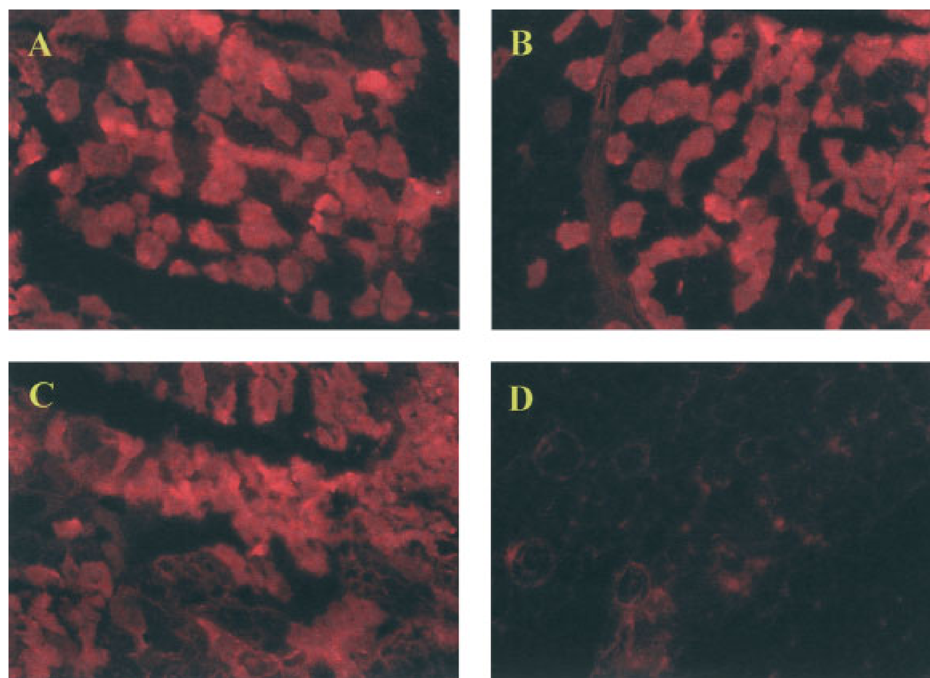

Figure 4. Evans blue (0.1%, 100 μl) was intravenously injected into mice followed by delivery of 6 LV pulses using the caliper electrode to the quadriceps with consistent (A) or alternating (B) polarity and either 200 V/cm, 20 ms pulses (C) or no pulses (D). Evans Blue fluorescence of 10 μm cryosections (1 day after the injection) from the quadriceps were examined by fluorescence microscopy at 200× magnification

Copyright © 2005 John Wiley & Sons, Ltd.

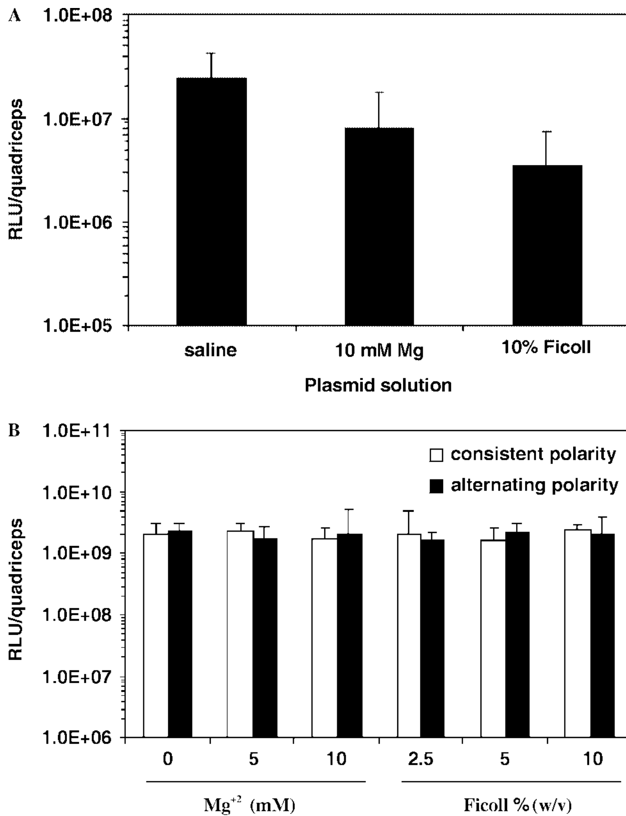

Figure 5. The levels of luciferase expression as a function of Mg^{2+} and Ficoll concentration in the medium. (A) Plasmid DNA in different media was injected into the muscle without electroporation. (B) The pulses (200 V/cm, 6 pulses, 20 ms), after DNA injection, were delivered by caliper electrodes with the consistent or alternating polarity. Data represent the mean ± SD (n = 3)

DNA enters cytoplasmic/endosomal compartments after electroporation, it may bind to cellular entities and might not be reversibly extracted. Thus a gradual buildup of the DNA in the cell still seems quite possible even under the condition of the rapid reversal of polarity. To rule out these possibilities, we designed an experiment in which DNA was injected (10 µg/10 µl) into the site between the epimysium (the fibrous sheath covering the muscle) and muscle fiber of quadriceps. Six pulses were delivered immediately after the injection of DNA. In this experiment, the current moves in a direction perpendicular to the length of the fiber, with one electrode on the epimysium directly over the site of injection and the other electrode on the opposite side of the muscle. When DNA is sandwiched between the epimysium and muscle, it can only move in two directions under the assumed electrophoretic force, either toward the muscle or toward the epimysium. The direction of this movement depends on the polarity of the pulses. If the electrophoretic force does in fact exist, the DNA should move toward the cells when the muscle is in contact with the anode of the caliper electrode (epimysium is in contact with the cathode) during electroporation (200 V/cm, one direction of six pulses). If this is the case, the efficiency of electro-gene transfer should be greatly enhanced. When the polarity is reversed (i.e. muscle is in contact with the cathode of the caliper electrode), the DNA would be driven away from the cells and thus, the efficiency of electro-gene transfer would dramatically decrease. The data in Figure 6 showed that very low levels of gene expression were observed for the control group (without electroporation) and more than 100-fold enhancement was detected for both electroporation groups. The enhancements were regardless of the polarity of the electrode. The levels of gene expression in Figure 6 were lower compared to that in Figure 2 because pDNA was not injected into the inside of muscle.

Discussion

For many years, the delivery of electric pulses has been used to introduce small molecules, or foreign genes, into prokaryotic and eukaryotic cells *in vitro* and *in vivo*. In previous *in vitro* studies, it was reported that the electrophoretic effects of an electric field on polyanionic DNA were likely to be of importance for efficient DNA transfer [25,30,31]. Electrophoresis could play a role [23] in several ways: (i) improving diffusion of DNA in the tissue; (ii) allowing DNA to have a closer contact with cell membrane; or (iii) inserting DNA into or driving DNA through the permeabilized membrane. The involvement of electrophoresis in electro-gene transfer was first reported by an *in vitro* experiment in which the transfection efficiency of cells on a monolayer porous film was found to vary, depending on the polarity of the pulse [25]. A single pulse (field intensity of 1500 V/cm, and duration 100 µs) with a polarity inducing DNA electrophoresis toward the cells resulted in a 10-fold excess of transfection efficiency compared to a pulse with reversed polarity [25]. It can be imagined that the reversed polarity with such a strong field,

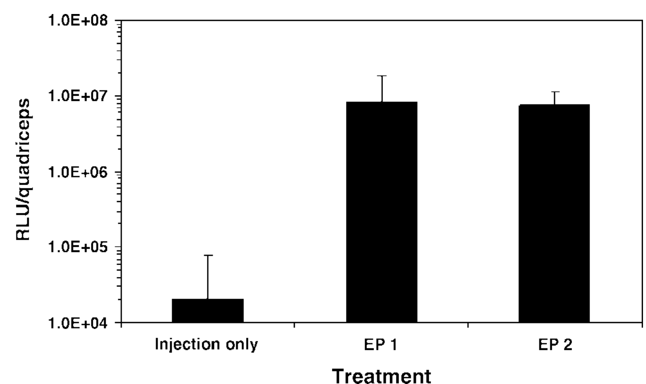

Figure 6. The level of luciferase expression after DNA was injected into the site between the epimysium and muscle fiber of quadriceps, followed by no electroporation (injection only), electroporation 1 (EP1) and electroporation 2 (EP2) using caliper electrodes. Data represent the mean ± SD (n = 3). EP1: epimysium was in contact with the cathode during electroporation (200 V/cm, 20 ms/pulse, one direction of six pulses). EP2: epimysium was in contact with the anode

Copyright © 2005 John Wiley & Sons, Ltd.

1500 V/cm, would induce the electrophoretic force which could drive DNA away from the cells, resulting in low transfection efficiency. We agree that there is an electrophoretic force in *in vitro* DNA transfer because the cell culture medium is electrically conductive. Before the electric pulses, DNA is randomly distributed between the electrodes, but, during the pulses, the molecules are then reoriented and drawn toward to the anode. The question is whether electrophoresis can drive DNA across the cell membrane, particularly in an *in vivo* situation.

Efficient procedures for DNA electro-transfer *in vivo* have been used for nearly a decade. However, the implied mechanism is still much unknown. It is generally accepted that electric pulses could induce electrophoresis, which is critical for *in vivo* gene transfer [3,21–23], although there is a lack of direct evidence to support the hypothesis. The theory for the DNA electrophoresis was proposed that when pores in the membrane are formed, electric-field lines concentrate in the pores [25]. The high field intensity in the pores will result in plasmid orientation and a subsequent fast passing of DNA through the pores [25]. A study favoring the hypothesis examined electro-gene transfer in the skeletal muscle using a combination of short HV (high voltage) and long LV (low voltage) pulses [21,23]. While a highly permeabilizing sequence of eight HV pulses led to a low level of gene transfer, the combination of one HV pulse followed by four LV pulses resulted in optimal gene transfer efficiency [21,23]. It was assumed that HV pulses could affect the cells (membrane permeabilization) but not promote significant electrophoresis because of their short duration, and that DNA transfer was facilitated by electrophoresis of the DNA induced by the LV pulses [21,23]. Another study supporting the hypothesis found that DNA had to be injected in muscles before the delivery of electric pulses to achieve enhanced gene transfer [3]. Mir *et al*. found, using skeletal muscle as a model, that adding ^{51}Cr-EDTA shortly after the delivery of electric pulses resulted in the uptake of ^{51}Cr-EDTA, whereas gene transfer did not occur when DNA was added after the pulses [3]. While the above observations are important for understanding electro-gene transfer, the data presented in our study indicated that electro-gene transfer *in vivo* is not facilitated by DNA electrophoresis. Efficient gene transfer by electroporation is associated with both cell membrane permeabilization and passive diffusion of DNA through the permeabilized membrane defect. An implication for these results is the ability to transfer neutral particles, such as PEGylated compounds, by electroporation.

Acknowledgements

This work was supported by National Institutes of Health Grants DK065964 (to F. Liu) and A148851 (to L. Huang).

References

1. Wolff JA, Malone RW, Williams P, *et al*. Direct gene transfer into mouse muscle in vivo. *Science* 1990; **247**: 1465–1468.
2. Nichols WW, Ledwith BJ, Manam SV, *et al*. Potential DNA vaccine integration into host cell genome. *Ann N Y Acad Sci* 1995; **772**: 30–39.
3. Mir LM, Bureau MF, Gehl J, *et al*. High-efficiency gene transfer into skeletal muscle mediated by electric pulses. *Proc Natl Acad Sci U S A* 1999; **96**: 4262–4267.
4. Wells DJ. Gene therapy progress and prospects: Electroporation and other physical methods. *Gene Ther* 2004; **11**: 1363–1369.
5. Andre F, Mir LM. DNA electrotransfer: its principles and an updated review of its therapeutic applications. *Gene Ther* 2004; **11**(Suppl 1): S33–42.
6. Ugen KE, Heller R. Electroporation as a method for the efficient in vivo delivery of therapeutic genes. *DNA Cell Biol* 2003; **22**: 753.
7. Liu F, Huang L. A syringe electrode device for simultaneous injection of DNA and electrotransfer. *Mol Ther* 2002; **5**: 323–328.
8. Dona M, Sandri M, Rossini K, *et al*. Functional in vivo gene transfer into the myofibers of adult skeletal muscle. *Biochem Biophys Res Commun* 2003; **312**: 1132–1138.
9. Sakai M, Nishikawa M, Thanaketpaisarn O, *et al*. Hepatocyte-targeted gene transfer by combination of vascularly delivered plasmid DNA and in vivo electroporation. *Gene Ther* 2005; **12**: 607–616.
10. Byrnes CK, Malone RW, Akhter N, *et al*. Electroporation enhances transfection efficiency in murine cutaneous wounds. *Wound Repair Regen* 2004; **12**: 397–403.
11. Dean DA, Machado-Aranda D, Blair-Parks K, *et al*. Electroporation as a method for high-level nonviral gene transfer to the lung. *Gene Ther* 2003; **10**: 1608–1615.
12. Marti G, Ferguson M, Wang J, *et al*. Electroporative transfection with kgf-1 DNA improves wound healing in a diabetic mouse model. *Gene Ther* 2004; **11**: 1780–1785.
13. Jeong JG, Kim JM, Ho SH, *et al*. Electrotransfer of human il-1ra into skeletal muscles reduces the incidence of murine collagen-induced arthritis. *J Gene Med* 2004; **6**: 1125–1133.
14. MacColl GS, Goldspink G, Bouloux PM. Using skeletal muscle as an artificial endocrine tissue. *J Endocrinol* 1999; **162**: 1–9.
15. Danko I, Fritz JD, Jiao S, *et al*. Pharmacological enhancement of in vivo foreign gene expression in muscle. *Gene Ther* 1994; **1**: 114–121.
16. Lu QL, Bou-Gharios G, Partridge TA. Nonviral gene delivery in skeletal muscle: a protein factory. *Gene Ther* 2003; **10**: 131–142.
17. Bigey P, Bureau MF, Scherman D. In vivo plasmid DNA electrotransfer. *Curr Opin Biotechnol* 2002; **13**: 443–447.
18. Fattori E, La Monica N, Ciliberto G, *et al*. Electro-gene-transfer: a new approach for muscle gene delivery. *Somat Cell Mol Genet* 2002; **27**: 75–83.
19. Neumann E, Kakorin S, Toensing K. Fundamentals of electroporative delivery of drugs and genes. *Bioelectrochem Bioenerg* 1999; **48**: 3–16.
20. Somiari S, Glasspool-Malone J, Drabick JJ, *et al*. Theory and in vivo application of electroporative gene delivery. *Mol Ther* 2000; **2**: 178–187.
21. Satkauskas S, Bureau MF, Puc M, *et al*. Mechanisms of in vivo DNA electrotransfer: Respective contributions of cell electropermeabilization and DNA electrophoresis. *Mol Ther* 2002; **5**: 133–140.
22. Miklavcic D, Semrov D, Mekid H, *et al*. A validated model of in vivo electric field distribution in tissues for electrochemotherapy and for DNA electrotransfer for gene therapy. *Biochim Biophys Acta* 2000; **1523**: 73–83.
23. Bureau MF, Gehl J, Deleuze V, *et al*. Importance of association between permeabilization and electrophoretic forces for intramuscular DNA electrotransfer. *Biochim Biophys Acta* 2000; **1474**: 353–359.
24. Matsuda R, Nishikawa A, Tanaka H. Visualization of dystrophic muscle fibers in mdx mouse by vital staining with evans blue: Evidence of apoptosis in dystrophin-deficient muscle. *J Biochem (Tokyo)* 1995; **118**: 959–964.
25. Klenchin VA, Sukharev SI, Serov SM, *et al*. Electrically induced DNA uptake by cells is a fast process involving DNA electrophoresis. *Biophys J* 1991; **60**: 804–811.

Copyright © 2005 John Wiley & Sons, Ltd.

26. Faurie C, Phez E, Golzio M, *et al*. Effect of electric field vectoriality on electrically mediated gene delivery in mammalian cells. *Biochim Biophys Acta* 2004; **1665**: 92–100.
27. Golzio M, Rols MP, Teissie J. In vitro and in vivo electric field-mediated permeabilization, gene transfer, and expression. *Methods* 2004; **33**: 126–135.
28. Golzio M, Teissie J, Rols MP. Cell synchronization effect on mammalian cell permeabilization and gene delivery by electric field. *Biochim Biophys Acta* 2002; **1563**: 23–28.
29. Faurie C, Golzio M, Moller P, *et al*. Cell and animal imaging of electrically mediated gene transfer. *DNA Cell Biol* 2003; **22**: 777–783.
30. Sukharev SI, Klenchin VA, Serov SM, *et al*. Electroporation and electrophoretic DNA transfer into cells. The effect of DNA interaction with electropores. *Biophys J* 1992; **63**: 1320–1327.
31. Kimoto H, Taketo A. Initial stage of DNA-electrotransfer into *E. coli* cells. *J Biochem (Tokyo)* 1997; **122**: 237–242.

Copyright © 2005 John Wiley & Sons, Ltd.