Expert Opinion

Electroporation-mediated gene therapy

Yoshitaka Isaka† & Enyu Imai
†*Osaka University Graduate School of Medicine, Departments of Advanced Technology for Transplantation and Nephrology, Suita, Osaka 565-0871, Japan*

Non-viral gene transfer is markedly enhanced by the application of *in vivo* electroporation. Electroporation is a safe and efficient system to introduce genes to a wide variety of tissues, including skeletal muscle, tumors, kidney, liver and skin. Electroporation has been demonstrated to be effective in numerous disease models. This review focuses on the principles of electroporation and the target tissues employed for gene therapy. Based on the accumulation of positive results, the first clinical study for the treatment of malignant melanoma is now underway, and preclinical studies have suggested that electroporation is useful as a gene therapy protocol.

Keywords: cytokine, DNA vaccine, electroporation, erythropoietin, IL-12

Expert Opin. Drug Deliv. (2007) 4(5):561-571

1. Introduction

Gene therapy in human beings was first performed in a girl with adenosine deaminase deficiency in 1990 [1]. In the 15 years since the first clinical trial, 1314 protocols for human gene therapy have been approved (updated in January 2007 [201]). Safe and efficient delivery of DNA *in vivo* is a major requirement for gene therapy applications. So far, 18% (n = 234) vectors used in gene therapy clinical trials are naked/plasmid DNA vectors. Traditionally, plasmid-based technology has been limited in scope because expression levels following naked DNA transfer have been low. However, numerous investigators have outlined the safety and toxicological concerns of injecting viruses for delivery of transgenes into animals and humans [2]. When compared with viral vectors, the advantages of naked DNA transfer include reduced potential for immunogenicity, integration into the genome and environmental spread. Although plasmid DNA or oligonucleotide-based gene transfer methods are attractive, efficient and/or effective delivery methods are lacking. However, one method that has emerged as a means to facilitate the delivery of plasmid DNA is *in vivo* electroporation.

Electroporation originated for *in vitro* transfection [3] has become a standard laboratory method. The discharge of an electric field changes the plasma membrane permeability, and thereby results in the uptake of molecules [4-6]. The application of *in vivo* electroporation for drug delivery was first demonstrated in a variety of different tumors in animals and humans [7]. This technique was then applied to plasmid DNA transfer [8,9]. *In vivo* electroporation is applicable to almost all tissues tested, with the primary issue being accessibility. The use of *in vivo* electroporation for plasmid DNA delivery has seen tremendous growth, including the initiation of clinical trials (e.g., 'Phase I Trial of Intratumoral pIL-12 Electroporation in Malignant Melanoma').

The increased use of *in vivo* electroporation for gene delivery has established its potential for numerous therapeutic applications. Numerous published studies describe the *in vivo* electroporation delivery of plasmid DNA. This review focuses on the most recent studies using *in vivo* electroporation for the delivery of plasmid

informa
healthcare

DNA or nucleotides, demonstrating a clear therapeutic response with the potential for clinical use.

2. Mechanism of electroporation

A more efficient level of plasmid DNA transfer and transgene expression can be accomplished by using a series of square-wave electric pulses to drive naked DNA into a stable, non-dividing population of cells. However, the exact mechanism of electroporation is largely unknown. The cell membrane, normally not permeable to large molecules such as DNA is thought to be equivalent to an electrical capacitor [10]. The physical process of electroporation exposes the target tissue to brief electric field pulses that induce a temporary and reversible breakdown of the cell membrane and the formation of pores [11]. In 1965, Coster reported the 'punch-through' phenomenon in membranes exposed to an electric field [12]. In 1973, Zimmermann *et al.* described a reversal electrical breakdown of the cell membrane [13]. They discharged electric pulses into an *Escherichia coli* suspension in a chamber and analyzed the size fractionation. They found a reduced volume of *E. coli* if a critical field strength is exceeded; thus suggesting that the cell membrane was breaking down. Zimmmerman thus proposed the following mechanism of electrical breakdown of the cell membrane *in vitro* [6]. As the cell membrane is extremely conductive, the current lines pass through the inside of the cell based on the increase in field strength. Consequently, pore formation by electrical breakdown occurs. This pore formation is very rapid, occurring within 10 ns [14], and the size of these pores was estimated to be < 10 nm [15,16]. With an increased duration of the electric field, the number of pores increases, and they merge. When the pores merge into a single large pore, irreversible breakdown occurs. The reclosing of the membrane can be delayed by keeping the cells at a low temperature. A smaller molecule, such as oligonucleotides can be transferred into the cytosol through the pore. However, DNA of at least 150 kb, which is larger than the pore, can be introduced by electroporation [17], suggesting that the mechanism of gene migration into cytosol is simply based on diffusion.

Thus, there is a question over how DNA diffuses across the permeabilized membrane: through a passive mechanism or through the effects of electrophoretic force? Mir *et al.* investigated the mechanisms of *in vivo* gene transfer by comparing *LacZ* gene expression and the uptake of ^{51}Cr-EDTA, a marker of small molecule uptake in muscle-fiber permeabilitzation [11]. Although the uptake of ^{51}Cr-EDTA appeared even when administered 30 s after an electric pulse, *LacZ* gene expression occurred only when administered before an electric pulse. These observations suggest that there are different mechanisms involved between DNA and small molecules which are uptaken under electric field exposure. The instability of the lipid bilayer and the electrophoreic forces induced by electric pulses may be critical for *in vivo* gene introduction [18-20]. Nevertheless, this hypothesis has been recently challenged [21] and a passive mechanism involving simple diffusion of DNA through the membranes was proposed. In addition, Chernomordik *et al.* proposed another hypothesis [22]. The electric pulse causes membrane instability and forms vesicles containing DNA, and thereafter the DNA is transferred to the cytosol by endocytosis. Xie *et al.* demonstrated that electric pulse treatment transferred only the DNA attached to the cell membrane [23]. Taken together, the electric pulses induce transfection not only by altering membrane permeabilization, but also by promoting DNA migration.

After electroporation, exogenous DNA is transcribed in the nucleus in a transient fashion and is rarely integrated into the genome. The frequency of mutations after electroporation is reported to be lower than after a $CaPO_4$ or diethylaminoethyl dextran co-precipitation method [24]. Although the actual mechanism remains controversial, numerous studies have focused on the practical aspects of electroporation, for example, the optimal conditions for nucleic acid delivery to ensure long-term high transgene expression levels, without causing pain or tissue damage.

3. Tissue-targeted gene transfer

Since the *in vivo* application of electroporation, the optimal conditions of electroporation (including the shape of the electrical pulse, pulse amplitude, pulse length, and electrode devices) have been examined in many tissues. Although many organs and tissues have been electroporated, most of these studies used skeletal muscle and tumors as the target organ.

3.1 Skeletal muscle

Since the initial report by Aihara and Miyazaki [25], *in vivo* electroporation of skeletal muscle for the delivery of therapeutic proteins has become widely used. Although the equipment, electroporation conditions, delivery methods, vectors and animal models differ substantially, all studies conclude that intramuscular plasmid injection followed by electroporation can be successfully used to deliver therapeutic genes. The muscle is the optimal target organ for plasmid DNA injection combined with electroporation, as the persistent, long-term production and secretion of proteins into the bloodstream can be expected. The postmitotic nature of muscle fibers, combined with their excellent vascularization, relatively easy access to numerous groups of muscle and the potential for localized expression, makes muscle a good target for gene therapy. Expression levels are increased by as much as two to three orders of magnitude over plasmid injection alone, to levels comparable to those of viral-mediated transfection, and physiological or therapeutic plasma levels of the gene product can be achieved.

Muscle fibers are postmitotic cells that have a long lifespan, and, thus, long-term transduction in muscle can be expected. The duration of gene expression is reported to be 9 – 19 months after electroporation-mediated *in vivo* gene transfer into skeletal muscle [26-28]. Reported studies using

electroporation-mediated gene transfer provide evidence that sustained levels of secreted molecules can be achieved using plasmids in a simple, safe and efficient manner, with significant potential for recombinant protein and vaccination therapies. Muscle can be used as an endocrine tissue to secrete proteins at therapeutic concentrations to circulate around the body. For example, erythropoietin in anemia or β-thalassemia, cytokines for the treatment of chronic diseases, or clotting factors in hemophilia. Electroporation was also recently recognized as a reliable method to enhance DNA vaccine delivery [29-31].

3.1.1 Supplementation

Skeletal muscle-targeting electroporation is suited to the supplementation of secretory molecules, such as erythropoietin, GM-CSF and FLT3 ligand [32,33], and Factor VIII [34] or IX [26] in hemophilia. A variety of methods have been reported for experimental erythropoietin gene therapy. Rizzuto *et al.* demonstrated that electroporation-mediated erythropoietin gene transfer increased the production and secretion of recombinant protein from mouse skeletal muscle by > 100-fold [35]. Therapeutic erythropoietin levels were attainable in mice with a single injection of as little as 1 µg of plasmid DNA, and the increase in hematocrit was long-lasting. Furthermore, they achieved pharmacological regulation of vector expression through a tetracycline-inducible promoter. Electroporation-mediated erythropoietin gene transfer has been applied to the treatment of mice with β-thalassemia [36]. The authors found that this procedure induced very high hematocrit levels associated with increased erythropoietin in β-thalassemic mice when compared to disease control mice. In addition, erythropoietin transfection has also been successfully applied to animals with renal disease [37,38]. The technique is also applicable to non-human primates [39]. However, to avoid adverse effects, such as polycythemia or hypertension, controllable erythropoietin vectors are desired. In addition to tetracycline-sensitive promoters, it has also been accomplished with the mifepristone-sensitive GeneSwitch system [40,41]. Moreover, Maruyama *et al.* demonstrated that liver-targeted soluble human erythropoietin receptor gene transfer by tail-vein injection with hydrodynamics-based transfection is useful for neutralizing erythropoietin delivered by *in vivo* electroporation [42].

3.1.2 Vaccination

The principle of immunization is to induce the generation of memory T and B cells and the presence of neutralizing antibody in the serum following the injection of a foreign protein. The observation that direct *in vivo* gene transfer of recombinant DNA resulted in expression of protein *in situ* led to the development of DNA vaccines. DNA vaccination has been intensely studied as a means of generating immunity against antigens from infectious agents or tumors, as the method is simple, versatile and safe. When compared with other methods, the advantage of DNA vaccination is that the delivery of the antigen gene can easily be coupled to the delivery of any number of genes that modify the immune response. Introducing the gene encoding a protein directly into the skin [43] or muscle [44] of an animal elicits an immune response. In fact, plasmid-based vaccine injection is an attractive approach as it provides several advantages over present vaccines: i) a reduced need for live-attenuated pathogens (i.e., bacteria and viruses) or recombinant proteins; ii) plasmid DNA can be manufactured in a very cost-effective manner; and iii) plasmid DNA can be stored with relative ease. After plasmid DNA is introduced, the organ will produce the antigen inducing the immune response, thereby acting as the 'bioreactor' [45]. Moreover, antigen presentation occurs through the MHC class I or class II restricted pathways, and all arms of the immune response are activated (i.e., helper T cells, cytotoxic T lymphocytes and humoral immunity).

DNA vaccination has been effective in rodents, but the results have been less impressive in large animals and humans because of low or poorly reproducible gene transfer efficiency [29]. Therefore, multiple immunizations with high DNA doses are often required to achieve modest responses, particularly in primates [46]. One reason for the lack of efficacy of DNA vaccines in large animals and in initial human clinical trials seems to be inefficient uptake of DNA by muscular tissue, which differs between small and large animal species. It has been demonstrated that the level of antibodies produced is directly related to antigen expression levels [47,48]. Consequently, many approaches have been investigated to improve these vaccines (reviewed in [49]), and one of the most effective has been *in vivo* electroporation. Although increased transfection efficiency and concomitant increased antigen expression may explain the increased immune response in animals treated with DNA injection and electrotransfer [46], damage to muscle cells and the release of 'danger signals' after electroporation may also contribute [30]. The work of Gronevik *et al.* [50], for example, supports the notion that tissue injury is relevant. They found that in mice immunized against human secreted alkaline phosphatase by combined intramuscular DNA inoculation and electroporation, the highest levels of antibody production occurred in mice with the most muscle damage. Several recent studies have confirmed the enhanced efficiency of electrotransfer in DNA immunization: after electrotransfer of a plasmid encoding a surface antigen of the hepatitis B virus, antibody titers increased in mice, rabbits and guinea-pigs [46]. This was also demonstrated in mice after the electrotransfer of a plasmid encoding a tuberculosis protein [51]. For example, a single injection of plasmid encoding neuraminidase from influenza virus followed by electroporation in mice was able to provide long-term protection from influenza [52]. The ability of DNA vaccines to break tolerance has found applications in tumor immunology because most tumor-associated antigens are poorly immunogenic self-molecules. For example, several studies have demonstrated the effectiveness of electroporation in

vaccination against melanoma-associated antigens, HER2/neu (c-ErB2) and carcinoembryonic antigen (CEA) [49].

3.1.3 *Cytokines or growth factors*

Regulatory cytokines such as TGF-β1, IL-4 and IL-10 can protect against several autoimmune or inflammatory diseases. The *in vivo* delivery of these cytokines can be performed by both viral and nonviral gene therapy methods [53,54]. However, cytokines have pleiotropic effects, for example, TGF-β1 is a fibrogenic growth factor in several diseases. Therefore, their administration can be associated with undesirable effects. On the other hand, cytokine inhibitors have the advantages of being non-toxic and long-lived in body fluids, as compared with most cytokines. Lawson *et al.* demonstrated that the gene transfer of an IFN-γ receptor/Fc (IFN-γR/Fc) fusion protein protected MRL-Fas^{lpr} mice from early death and reduced autoantibody titers, renal disease and histological markers of a systemic lupus erythematosus-like disease [55]. In addition, electroporation after intramuscular injection of plasmid DNA coding a human IL-1 receptor antagonist [56] or soluble TNF receptor-Fc [57] in a collagen-induced arthritis model resulted in significant inhibition of collagen-induced arthritis (CIA). An experimental model of glomerulonephritis was ameliorated by interfering PDGF using PDGF receptor/Fc chimeric gene transfer into muscle [58], and ciclosporin-induced nephrotoxicity was suppressed by TGF-β receptor/Fc chimeric gene transfer [59].

In contrast, wound healing in diabetic mice is enhanced by the delivery of plasmids encoding TGF-β [60], although gene transfer encoding a TGF-β antagonist has been shown to ameliorate kidney disease. Growth factor gene transfer may also be useful in reducing the adverse effects associated with therapeutic agents. The delivery of plasmids encoding growth factors has been shown to suppress the toxicity associated with carbon tetrachloride intoxication [61], or with the clinical use of ciclosporin A [62], bleomycin [63] or cisplatin [64].

3.2 Tumors

Electroporation-mediated chemotherapy, or enhanced delivery of chemotherapeutic drugs, particularly bleomycin, and more recently cisplatin [65], to accessible solid tumors has been successfully used in humans for many years [66,67]. Recently, investigators focused on intratumoral plasmid delivery as a means to increase long-term antitumor immunity [68,69], to inhibit angiogenesis [70] or to reduce tumor volume [71]. Studies have shown that the intratumoral electrotransfer of genes is more effective than intramuscular electrotransfer of the same gene in the eradication of established tumors [72], and this difference is explained by underlying immunological mechanisms, T-cell infiltration, cytotoxic T lymphocyte activity and direct inhibition of angiogenesis [72].

Suicide gene therapy using herpes simplex virus-thymidine kinase/ganciclovir technology has suppressed the growth and metastasis of subcutaneously grafted mammary tumors in mice, although no complete regression was noted [71]. Significant inhibition of tumor growth was also obtained with intratumoral electroporation of TNF-related apoptosis-inducing ligand (TRAIL/Apo2 ligand), an apoptosis inducer [73]. Some authors have also shown that intratumoral electroporation of antisense MBD2, an enzyme involved in DNA methylation, results in significant inhibition of tumor growth in a nude mouse graft human tumor model [74]. The electroporation of plasmids encoding cytokines into tumors has been widely used. IFN-α [75], IL-12 and IL-18 [76] have been shown to reduce tumor growth and increase survival times in different tumor models.

3.3 Kidney

The combination of local DNA injection in muscle or tumor followed by *in vivo* electroporation has been shown to result in highly efficient gene expression, but gene expression was only observed in the limited area where the injected DNA was distributed. To apply this simple gene transfer technique to the treatment of various glomerular or tubulointerstitial diseases other than renal cancer, therapeutic genes should be introduced diffusely into glomeruli or tubulointersitium. To overcome this problem, Tsujie *et al.* succeeded in glomerular mesangial-targeted gene transfer by electroporation combined with selective infusion of DNA into the renal artery [77]. They employed a pair of tweezer-type electrodes to administer electric pulses into the whole kidney. As glomerular mesangial cells react to injury and secrete various inflammatory cytokines and growth factors, mesangium-targeting therapies have been reported. Electroporation-mediated transfection of a DNAzyme for TGF-β into glomerular mesangial cells suppressed TGF-β expression, thereby inhibiting consequent glomerular extracellular matrix accumulation in anti-Thy-1 nephritic kidneys [78]. RNA interference (RNAi), which is initiated by the introduction of dsRNA into the cell, leads to sequence-specific destruction of endogenous RNA. Takabatake *et al.* proved that electroporation is also effective in RNAi transfection *in vivo*. They showed that the electrotransfer of short interference RNA (siRNA) against TGF-β1 into mesangial cells significantly ameliorated glomerular matrix expansion in experimental glomerulonephritis [79]. Glomerular extracellular matrix expansion was also ameliorated by overexpression of *ets-1* [80] or hepatocyte growth factor [81] introduced by electroporation.

Because tubulointerstitial inflammation and fibrosis are common features in a variety of progressive renal diseases, and may indicate the degree of impairment of renal function and predict long-term prognosis more accurately than glomerular injury, targeting interstitial cells has proven to be challenging. Nakamura *et al.* demonstrated that electroporation combined with retrograde DNA infusion via the ureter resulted in the transfection into interstitial fibroblasts [82]. Of interest is whether retrograde infusion of DNA via the ureter allows the DNA to enter the interstitial area bypassing between papilla epithelial cells, after which DNA is distributed diffusely in the cortical interstitial spaces [83]. Furthermore, Fujii *et al.* demonstrated a simple electroporation method with high transfection efficiency and reduced surgical invasiveness [84]. The injection of DNA

directly into parenchyma followed by electroporation resulted in gene transfer into interstitial fibroblasts.

3.4 Liver

The liver is one of the primary targets for gene therapy in numerous metabolic diseases, cancers, hepatitis and other pathologies. Although recombinant viral vectors, particularly adenoviral vectors, have been widely used to introduce new genes into the liver, their usefulness may be mitigated by side effects, potential safety concerns and immunologic reactions to viral components, which often precludes redosing [85-88]. Plasmids delivered to the hepatic vasculature [89] by hydrodynamic methods [90,91] have been reported to deliver transgenes to the liver; however, hydrodynamic methods often require injection of large volumes of fluid, thus preventing application to humans. Electroporation combined with chemotherapy (electrochemotherapy) has been performed for different malignancies, including liver malignancies in animal models [92]. Recently, tumor reduction was successful with intratumoral IL-12 gene therapy by electroporation [93]. In addition, transgene expression driven by the phosphoenolpyruvate carboxykinase gene promoter is nutritionally regulated in the liver by this method [94], and hydrodynamic methods have recently improved. When combining intravenous reporter gene plasmid injection with electroporation, expression levels were shown to increase up to 200,000-fold when compared with plasmid injection alone [95]. The strategy of RNAi-based gene silencing in combination with electroporation also provides a possible complement to the limited therapeutic options currently available for chronic hepatitis B or C virus infection [96].

3.5 Skin

Electroporation-mediated plasmid DNA delivery to the skin is promising as an alternative technique for DNA vaccination and other therapeutic gene transfer applications requiring local or systemic delivery of recombinant protein [97]. Direct plasmid injection into the skin typically results in transduction restricted to the epidermis. However, in different animal species, when electroporation is applied after the injection, larger numbers of adipocytes and fibroblasts and numerous dendritic-like cells within the dermal and subdermal tissues, as well as lymph nodes draining electropermeabilized sites, are transfected [98,99]. The delivery of therapeutic genes, such as keratinocyte growth factor [100] or TGF-β [60], has been shown to improve wound healing in a mouse wound-healing model. DNA vaccines, such as plasmids expressing the hepatitis B surface antigen can be efficiently produced by skin cells and can elicit humoral and cellular responses [101] following plasmid injection and electroporation.

3.6 Other organs

Numerous other tissues or organs have been successful targets for electroporation-mediated gene transfer. Electroporation has been also employed to transfer genes to the retina [102-104]. A mild electric pulse (99 ms-long pulses of 12 V/cm strength) resulted in high levels of gene expression without causing serious damage [105]. For *in vivo* electroporation in the living eyeball, the eyeball is placed between the two electrode discs to administer electric pulses immediately after the intravitreous injection of DNA [105].

Several organs or tissues, such as vasculature, airways, and intestinal smooth muscle cells, cannot be directly accessed to deliver DNA, but genes could be delivered from the inside. In one study, intra-tracheal administration of *HGF* gene followed by electroporation transduced bronchial and alveolar epithelial cells, thereby reducing bleomycin-induced pulmonary fibrosis [106]. Although several electroporation approaches have been reported, a porous balloon catheter-mediated electroporation system is an elegant method. The electrode system uses the guide wire as one electrode and an internal wrapped wire contained within the balloon as the other electrode [107]. This transluminal catheter-based electroporation was shown to induce transduction in vasucular subendothelial cell layers in pigs, suggesting that transcutaneous intraluminal electroporation-mediated gene therapy may be applicable. Bladder smooth muscle has been also transduced by electroporation-mediated transfection by injecting the DNA into the subserosal region of the exposed bladder wall [108].

3.7 Clinical trials

Although recombinant IL-12 (rIL-12) therapy had some success for the treatment of cancer, systemically administered rIL-12 induces serious side effects [109]. Rakhmilevich *et al.* compared the antitumor efficacy and side effects of an *in vivo* IL-12 transfer and systemic rIL-12 therapy [110]. Although the antitumor efficacy of rIL-12 therapy appeared to be slightly higher than that of IL-12 gene therapy in B16 melanoma models, IL-12 gene therapy resulted in reduced mortality levels in mice. Recent Phase I melanoma clinical trials using naked IL-12 plasmid showed some beneficial clinical effects [111]. Recent preclinical studies suggest that electroporation can dramatically increase the efficacy of IL-12 gene therapy [68]. In addition, no significant toxic effects due to electroporation-mediated IL-12 gene therapy were observed in B16 mouse melanoma models [112]. Together with these observations, Phase I melanoma clinical trials by electroporation-mediated IL-12 intratumoral gene therapy has started to determine the side effects, the correct dose of treatment and its effectiveness in treating melanoma.

4. Conclusion

In this review, the authors have shown that gene transfer using electroporation can be effectively applied in both small and large animals, and this approach has been employed in clinical human gene therapy. Numerous studies have demonstrated the effectiveness of intratumoral delivery of therapeutic genes. In addition, DNA vaccination has been

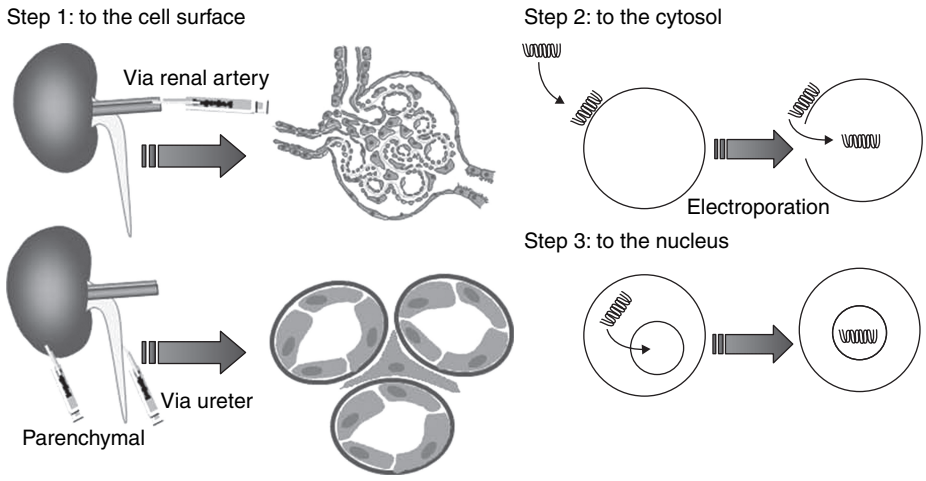

Figure 1. Three steps in gene therapy. Step 1: the administration route to deliver the therapeutic gene to the surface of the target cell should be selected. For example, in the kidney, the administration of DNA via renal artery results in transduction in glomerular mesangial cells, while parenchymal injection or ureteral injection leads to transfection into interstitial cells. In addition, augmentation of intrinsic hydrostatic organ pressure may distribute plasmid DNA diffusely to the cell surface. Step 2: electroporation enhances the efficacy of the second step, which transfers the DNA from cell surface to the cytosol. Step 3: Transfected DNA is carried to the nucleus, and thereafter transduced into the nucleus.

reported to be effective against antigens from various sources, such as cancer, infection and inflammatory diseases. The systemic delivery of therapeutic molecules such as erythropoietin by transferring genes into muscle or skin is also promising. For electroporation, surface tissues or organs, such as skin, muscle or subcutaneous tumors, are apparently suitable. However, in animal models, internal tissues, such as kidney, liver or internal tumors, are also a good targets for electroporation. To apply electroporation-mediated gene therapy to an internal organ, it will be important to develop suitable electrodes for effective delivery. Based on the accumulation of positive results, the first clinical study for the treatment of malignant melanoma has begun. Preclinical studies have suggested that electroporation is useful for gene therapy. Although clinical trials have already begun, there are numerous limitations that must be overcome before routine clinical use. Nevertheless, future research is expected to yield site- and disease-specific electroporation delivery strategies and to overcome present limitations.

5. Expert opinion

Gene therapy was originally a clinical strategy in which genetic material was transferred to somatic cells in order to correct an inherited genetic disorder. Gene therapy provides: i) correction of cellular dysfunction by expressing the deficient gene; ii) the addition of new cellular functions by the transfer of an exogenous gene; and iii) inhibition of unfavorable cellular actions by introducing a counteracting gene. Thus, the application of gene therapy has been extended to the treatment of acquired diseases, including cancer, infections and vascular diseases. The success of gene therapy is strongly dependent on the use of an efficient gene transfer system to allow the expression of the therapeutic gene in a specific organ, tissue or cell. In general, gene therapy is actually a three-step process (**Figure 1**): i) carrying the therapeutic gene to the surface of the target cells; ii) introducing it into cells; and iii) incorporating it into the nucleus. Although electroporation enhances the efficacy of the second phase, sufficient attention should also be paid to the first step. As mentioned in Section 2, therapeutic genes should be delivered to the surface of the target cell prior to the administration of electric pulses. We believe that the augmentation of intrinsic hydrostatic organ pressure may distribute plasmid DNA diffusely to the cell surface, and thereby result in increased transduction. In addition, an accessible route for site-specific transfection should be selected because different administration routes determine the transduced cells in some organs, such as in the kidney.

Although delivery systems using electroporation have been investigated, the optimal delivery conditions need to be examined. For example, electrical parameters such as voltage, pulse width, the number and frequency, as well as electrode configuration/instrumentation, should be determined for the target tissue. Electrode configuration is also very important, particularly for plate- versus and needle-type electrodes. In the case of murine muscle, tranduction efficacy by electroporation seems to be better in pretibial muscle than in gastrocnemius muscle, and tearing the muscle capsule decreases transfection efficiency. In the case of parenchymal kidney transfection, DNA injection with the clamping of renal vessels followed by electroporation has resulted in global interstitial transduction [84]. In the case of renal transfection via the renal artery [77] or ureter [82], a slight increase in hydrodynamic pressure augments transduction, suggesting that the stress of slight increases in intrinsic renal pressure might induce

global distribution, thereby increasing transfection efficiency. Therefore, we should examine the optimal delivery conditions of plasmid DNA injection. For example, the route of DNA delivery, the volume of injected DNA, the speed of injection and the clamping of the draining vessels should all be determined according to the target tissue.

Gene transfer by electroporation, which uses plasmid DNA as the vector, has several advantages over the conventional gene transfer method using viral vectors. The preparation of a large quantity of highly purified plasmid DNA is easy and inexpensive. In addition, combination gene therapy can be performed easily and simply in the same manner as for the single-gene therapy, merely by mixing more than one therapeutic plasmids. Multiple plasmids can be introduced into the same cells by electroporation [103]. For example, electroporation-mediated co-transfection with an Epstein-Barr virus nuclear antigen-1 expression vector has been shown to enhance the transgene expression of an origin of latent viral DNA replication-harboring plasmid vector [77]. Recently, Matsuda and Cepko demonstrated an elegant strategy using the Cre/loxP system in combination with cell-type specific promoter-regulated recombination system to restrict transgene expression in specified cell types in retina [103]. Furthermore, co-transfection of cytokine genes could effectively modulate the immune responses in a large animal following *in vivo* electroporation of a DNA vaccine [113].

Although electroporation-mediated chemotherapy has been successfully used for many years, electrical shock may be the hurdle to further clinical applications. Transplanted organs may, therefore, be good candidates for electroporation-mediated gene therapy because electric pulses can be administered only to the donor organ *ex vivo*. By using this strategy, the reno-protective potential and safety of *HGF* gene transfer was assessed using a porcine kidney transplant warm ischemia injury model [114]. Following left porcine kidney removal, 10 mins of warm ischemic injury was intentionally induced. Next, the *HGF* expression vector was infused into the renal artery with the renal vein clamped *ex vivo*, and electric pulses were discharged using bathtub-type electrodes, which resulted in transfection into glomerular cells. Kidney grafts were then transplanted after removing the right kidney. Electroporation-mediated HGF transfection protected kidneys from initial injury followed by tubulointerstitial fibrosis at 6 months post-transplantation. Kobayashi *et al.* have also demonstrated successful *ex vivo* gene transfection via the portal vein following electroporation [115,116].

Gene expression by electroporation-mediated plasmid DNA transfer is transient and the control of the gene expression is desired. It has been elucidated that the silencing of transgene expression occurs, even though transgenes are retained in the cells. Various studies have shown that a decrease in transcription level, rather than elimination of the transfected gene, is responsible for this effect [117]. Recently, Fujii *et al.* demonstrated that the gene expression level was gradually decreased up to two orders of magnitude after 6 weeks, but that treatment with histone deacetylase inhibitor FR901228 restored it to near the initial level [84]. Although the molecular mechanism is still unknown, FR901228 has been shown to specifically enhance exogenous transgene expression *in vivo* [118]. These observations suggest that the decrease in transgene expression is associated with histone deacetylation, which could be recovered by treatment with histone deacetylase inhibitor. Therefore, repeated injection of histone deacetylase inhibitor may maintain long-term transgene expression [84]. In addition, combination suicide gene therapy with FR901228, which was first developed as an anticancer drug, may be applicable as a therapeutic regimen for the treatment of malignant tumor [118].

Bibliography

Papers of special notes have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. BLAESE RM, CULVER KW, MILLER AD *et al.*: T lymphocyte-directed gene therapy for ADA-SCID: initial trial results after 4 years. *Science* (1995) **270**(5235):475-480.
2. PILARO AM, SERABIAN MA: Preclinical development strategies for novel gene therapeutic products. *Toxicol. Pathol.* (1999) **27**(1):4-7.
3. NEUMANN E, SCHAEFER-RIDDER M, WANG Y, HOFSCHNEIDER PH: Gene transfer into mouse lyoma cells by electroporation in high electric fields. *EMBO J.* (1982) **1**:841-845.
   • **First application of electroporation for gene transfer *in vitro*.**
4. POTTER H: Electroporation in biology: methods, applications, and instrumentation. *Anal. Biochem.* (1988) **174**(2):361-373.
5. TSONG TY: Electroporation of cell membranes. *Biophys. J.* (1991) **60**(2):297-306.
6. ZIMMERMANN U: Electric field-mediated fusion and related electrical phenomena. *Biochim. Biophys. Acta* (1982) **694**(3):227-277.
7. GOTHELF A, MIR LM, GEHL J: Electrochemotherapy: results of cancer treatment using enhanced delivery of bleomycin by electroporation. *Cancer Treat. Rev.* (2003) **29**(5):371-387.
8. HELLER R, JAROSZESKI M, ATKIN A *et al.*: *In vivo* gene electroinjection and expression in rat liver. *FEBS Lett.* (1996) **389**:225-228.
9. NISHI T, YOSHIZATO K, YAMASHIRO S *et al.*: High-efficiency *in vivo* gene transfer using intraarterial plasmid DNA injection following *in vivo* electroporation. *Cancer Res.* (1996) **56**(5):1050-1055.
10. ZAMPAGLIONE I, ARCURI M, CAPPELLETTI M *et al.*: *In vivo* DNA gene electro-transfer: a systematic analysis of different electrical parameters. *J. Gene Med.* (2005) 7(11):1475-1481.
11. MIR LM, BUREAU MF, GEHL J *et al.*: High-efficiency gene transfer into skeletal muscle mediated by electric pulses. *Proc. Natl. Acad. Sci. USA* (1999) **96**(8):4262-4267.
    • **Evidence for intracellular transport mechanism by electroporation.**
12. COSTER HG: A quantitative analysis of the voltage-current relationships of fixed

charge membranes and the associated property of "punch-through". *Biophys. J.* (1965) **5**(5):669-686.

13. ZIMMERMANN U, SCHULZ J, PILWAT G: Transcellular ion flow in *Escherichia coli* B and electrical sizing of bacterias. *Biophys. J.* (1973) **13**(10):1005-1013.
• **Hypothesis for the electrical breakdown of cell membrane.**
14. BENZ R, ZIMMERMANN U: Pulse-length dependence of the electrical breakdown in lipid bilayer membranes. *Biochim. Biophys. Acta* (1980) **597**(3):637-642.
15. BENZ R, ZIMMERMANN U: The resealing process of lipid bilayers after reversible electrical breakdown. *Biochim. Biophys. Acta* (1981) **640**(1):169-178.
16. CHANG DC, REESE TS: Changes in membrane structure induced by electroporation as revealed by rapid-freezing electron microscopy. *Biophys. J.* (1990) **58**(1):1-12.
17. KNUTSON JC, YEE D: Electroporation: parameters affecting transfer of DNA into mammalian cells. *Anal. Biochem.* (1987) **164**(1):44-52.
18. BUREAU MF, GEHL J, DELEUZE V, MIR LM, SCHERMAN D: Importance of association between permeabilization and electrophoretic forces for intramuscular DNA electrotransfer. *Biochim. Biophys. Acta* (2000) **1474**(3):353-359.
19. SATKAUSKAS S, ANDRE F, BUREAU MF, SCHERMAN D, MIKLAVCIC D, MIR LM: Electrophoretic component of electric pulses determines the efficacy of *in vivo* DNA electrotransfer. *Hum. Gene Ther.* (2005) **16**(10):1194-1201.
20. 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**(2):133-140.
21. LIU F, HESTON S, SHOLLENBERGER LM *et al.*: Mechanism of *in vivo* DNA transport into cells by electroporation: electrophoresis across the plasma membrane may not be involved. *J. Gene Med.* (2006) **8**(3):353-361.
22. CHERNOMORDIK LV, SOKOLOV AV, BUDKER VG: Electrostimulated uptake of DNA by liposomes. *Biochim. Biophys. Acta* (1990) **1024**(1):179-183.
• **Hypothesis for vesicle formation by electroporation.**
23. XIE TD, SUN L, TSONG TY: Study of mechanisms of electric field-induced DNA transfection. I. DNA entry by surface binding and diffusion through membrane pores. *Biophys. J.* (1990) **58**(1):13-19.
• **Examination of mechanism of electroporation.**
24. DRINKWATER NR, KLINEDINST DK: Chemically induced mutagenesis in a shuttle vector with a low-background mutant frequency. *Proc. Natl. Acad. Sci. USA* (1986) **83**(10):3402-3406.
25. AIHARA H, MIYAZAKI J: Gene transfer into muscle by electroporation *in vivo* *Nat. Biotechnol.* (1998) **16**:867-870.
•• **Initial report of *in vivo* electroporation of muscle for delivery of therapeutic molecule.**
26. BETTAN M, EMMANUEL F, DARTEIL R *et al.*: High-level protein secretion into blood circulation after electric pulse-mediated gene transfer into skeletal muscle. *Mol. Ther.* (2000) **2**(3):204-210.
27. MURAMATSU T, ARAKAWA S, FUKAZAWA K *et al.*: *In vivo* gene electroporation in skeletal muscle with special reference to the duration of gene expression. *Int. J. Mol. Med.* (2001) 7(1):37-42.
28. TONE CM, CARDOZA DM, CARPENTER RH, DRAGHIA-AKLI R: Long-term effects of plasmid-mediated growth hormone releasing hormone in dogs. *Cancer Gene Ther.* (2004) **11**(5):389-396.
29. BABIUK LA, PONTAROLLO R, BABIUK S, LOEHR B, VAN DRUNEN LITTEL-VAN DEN HURK S: Induction of immune responses by DNA vaccines in large animals. *Vaccine* (2003) **21**(7-8):649-658.
30. SCHEERLINCK JP, KARLIS J, TJELLE TE, PRESIDENTE PJ, MATHIESEN I, NEWTON SE: *In vivo* electroporation improves immune responses to DNA vaccination in sheep. *Vaccine* (2004) **22**(13-14):1820-1825.
31. WU CJ, LEE SC, HUANG HW, TAO MH: *In vivo* electroporation of skeletal muscles increases the efficacy of Japanese encephalitis virus DNA vaccine. *Vaccine* (2004) **22**(11-12):1457-1464.
32. PERETZ Y, ZHOU ZF, HALWANI F, PRUD'HOMME GJ: *In vivo* generation of dendritic cells by intramuscular codelivery of FLT3 ligand and GM-CSF plasmids. *Mol. Ther.* (2002) **6**(3):407-414.
33. SHIMAO K, TAKAYAMA T, ENOMOTO K *et al.*: Cancer gene therapy using *in vivo* electroporation of Flt3-ligand. *Intern. J. Oncol.* (2005) **27**(2):457-463.
34. LONG YC, JAICHANDRAN S, HO LP, TIEN SL, TAN SY, KON OL: FVIII gene delivery by muscle electroporation corrects murine hemophilia A. *J. Gene. Med.* (2005) 7(4):494-505.
35. RIZZUTO G, CAPPELLETTI M, MAIONE D *et al.*: Efficient and regulated erythropoietin production by naked DNA injection and muscle electroporation. *Proc. Natl. Acad. Sci. USA* (1999) **96**(11):6417-6422.
•• **Demonstration of supplementation therapy for serum protein deficiencies.**
36. PAYEN E, BETTAN M, ROUYER-FESSARD P, BEUZARD Y, SCHERMAN D: Improvement of mouse β-thalassemia by electrotransfer of erythropoietin cDNA. *Exp. Hematol.* (2001) **29**(3):295-300.
37. ATAKA K, MARUYAMA H, NEICHI T, MIYAZAKI J, GEJYO F: Effects of erythropoietin-gene electrotransfer in rats with adenine-induced renal failure. *Am. J. Nephrol.* (2003) **23**(5):315-323.
38. MARUYAMA H, ATAKA K, GEJYO F *et al.*: Long-term production of erythropoietin after electroporation-mediated transfer of plasmid DNA into the muscles of normal and uremic rats. *Gene Ther.* (2001) **8**(6):461-468.
39. FATTORI E, CAPPELLETTI M, ZAMPAGLIONE I *et al.*: Gene electro-transfer of an improved erythropoietin plasmid in mice and non-human primates. *J. Gene Med.* (2005) 7(2):228-236.
40. TERADA Y, TANAKA H, OKADO T *et al.*: Efficient and ligand-dependent regulated erythropoietin production by naked DNA injection and *in vivo* electroporation. *Am. J. Kidney Dis.* (2001) **38**(4 Suppl. 1):S50-S53.
41. TERADA Y, TANAKA H, OKADO T *et al.*: Ligand-regulatable erythropoietin production by plasmid injection and *in vivo* electroporation. *Kidney Int.* (2002) **62**(6):1966-1976.

42. MARUYAMA H, HIGUCHI M, HIGUCHI N *et al.*: Post-secretion neutralization of transgene-derived effect: soluble erythropoietin receptor/IgG1Fc expressed in liver neutralizes erythropoietin produced in muscle. *J. Gene Med.* (2004) **6**(2):228-237.

43. TANG DC, DEVIT M, JOHNSTON SA: Genetic immunization is a simple method for eliciting an immune response. *Nature* (1992) **356**(6365):152-154.

44. ULMER JB, DONNELLY JJ, PARKER SE *et al.*: Heterologous protection against influenza by injection of DNA encoding a viral protein. *Science* (1993) **259**(5102):1745-1749.

45. VAN DRUNEN LITTEL-VAN DEN HURK S, BABIUK SL, BABIUK LA: Strategies for improved formulation and delivery of DNA vaccines to veterinary target species. *Immunol. Rev.* (2004) **199**:113-125.

46. WIDERA G, AUSTIN M, RABUSSAY D *et al.*: Increased DNA vaccine delivery and immunogenicity by electroporation *in vivo*. *J. Immunol.* (2000) **164**(9):4635-4640.

•• **Mechanisms for electroporation-mediated DNA vaccines.**

47. KIRMAN JR, SEDER RA: DNA vaccination: the answer to stable, protective T-cell memory? *Curr. Opin. Immunol.* (2003) **15**(4):471-476.

48. LEE AH, SUH YS, SUNG JH, YANG SH, SUNG YC: Comparison of various expression plasmids for the induction of immune response by DNA immunization. *Mol. Cells* (1997) 7(4):495-501.

49. PRUD'HOMME GJ: DNA vaccination against tumors. *J. Gene Med.* (2005) 7(1):3-17.

50. GRONEVIK E, VON STEYERN FV, KALHOVDE JM, TJELLE TE, MATHIESEN I: Gene expression and immune response kinetics using electroporation-mediated DNA delivery to muscle. *J. Gene Med.* (2005) 7(2):218-227.

51. TOLLEFSEN S, VORDERMEIER M, OLSEN I *et al.*: DNA injection in combination with electroporation: a novel method for vaccination of farmed ruminants. *Scand. J. Immunol.* (2003) **57**(3):229-238.

52. CHEN J, FANG F, LI X, CHANG H, CHEN Z: Protection against influenza virus infection in BALB/c mice immunized with a single dose of neuraminidase-expressing DNAs by electroporation. *Vaccine* (2005) **23**(34):4322-4328.

53. PRUD'HOMME GJ, LAWSON BR, CHANG Y, THEOFILOPOULOS AN: Immunotherapeutic gene transfer into muscle. *Trends Immunol.* (2001) **22**(3):149-155.

54. PRUD'HOMME GJ, LAWSON BR, THEOFILOPOULOS AN: Anticytokine gene therapy of autoimmune diseases. *Expert Opin. Biol. Ther.* (2001) **1**(3):359-373.

55. LAWSON BR, PRUD'HOMME GJ, CHANG Y *et al.*: Treatment of murine lupus with cDNA encoding IFN-γR/Fc. *J. Clin. Investig.* (2000) **106**(2):207-215.

56. JEONG JG, KIM JM, HO SH, HAHN W, YU SS, KIM S: Electrotransfer of human IL-1Ra into skeletal muscles reduces the incidence of murine collagen-induced arthritis. *J. Gene Med.* (2004) **6**(10):1125-1133.

57. KIM JM, HO SH, HAHN W *et al.*: Electro-gene therapy of collagen-induced arthritis by using an expression plasmid for the soluble p75 tumor necrosis factor receptor-Fc fusion protein. *Gene Ther.* (2003) **10**(15):1216-1224.

58. NAKAMURA H, ISAKA Y, TSUJIE M *et al.*: Electroporation-mediated PDGF receptor-IgG chimera gene transfer ameliorates experimental glomerulonephritis. *Kidney Int.* (2001) **59**(6):2134-2145.

59. XIN J, HOMMA T, MATSUSAKA T *et al.*: Suppression of cyclosporine A nephrotoxicity *in vivo* by transforming growth factor β receptor-immunoglobulin G chimeric protein. *Transplantation* (2004) 77(9):1433-1442.

60. LEE PY, CHESNOY S, HUANG L: Electroporatic delivery of TGF-β1 gene works synergistically with electric therapy to enhance diabetic wound healing in db/db mice. *J. Invest. Dermatol.* (2004) **123**(4):791-798.

61. XUE F, TAKAHARA T, YATA Y *et al.*: Attenuated acute liver injury in mice by naked hepatocyte growth factor gene transfer into skeletal muscle with electroporation. *Gut* (2002) **50**(4):558-562.

62. MIZUI M, ISAKA Y, TAKABATAKE Y *et al.*: Electroporation-mediated HGF gene transfer ameliorated cyclosporine nephrotoxicity. *Kidney Intern.* (2004) **65**(6):2041-2053.

63. UMEDA Y, MARUI T, MATSUNO Y *et al.*: Skeletal muscle targeting *in vivo* electroporation-mediated HGF gene therapy of bleomycin-induced pulmonary fibrosis in mice. *Lab. Invest.* (2004) **84**(7):836-844.

64. PRADAT PF, FINIELS F, KENNEL P *et al.*: Partial prevention of cisplatin-induced neuropathy by electroporation-mediated nonviral gene transfer. *Hum. Gene Ther.* (2001) **12**(4):367-375.

65. REBERSEK M, CUFER T, CEMAZAR M, KRANJC S, SERSA G: Electrochemotherapy with cisplatin of cutaneous tumor lesions in breast cancer. *Anticancer Drugs* (2004) **15**(6):593-597.

66. BELEHRADEK M, DOMENGE C, LUBOINSKI B, ORLOWSKI S, BELEHRADEK J JR, MIR LM: Electrochemotherapy, a new antitumor treatment. First clinical Phase I – II trial. *Cancer* (1993) **72**(12):3694-3700.

•• **First clinical trail of electrochemotherapy.**

67. MIR LM, DEVAUCHELLE P, QUINTIN-COLONNA F *et al.*: First clinical trial of cat soft-tissue sarcomas treatment by electrochemotherapy. *Br. J. Cancer* (1997) **76**(12):1617-1622.

68. LUCAS ML, HELLER L, COPPOLA D, HELLER R: IL-12 plasmid delivery by *in vivo* electroporation for the successful treatment of established subcutaneous B16. F10 melanoma. *Mol. Ther.* (2002) **5**(6):668-675.

69. LUCAS ML, HELLER R: Immunomodulation by electrically enhanced delivery of plasmid DNA encoding IL-12 to murine skeletal muscle. *Mol. Ther.* (2001) **3**(1):47-53.

70. CICHON T, JAMROZY L, GLOGOWSKA J, MISSOL-KOLKA E, SZALA S: Electrotransfer of gene encoding endostatin into normal and neoplastic mouse tissues: inhibition of primary tumor growth and metastatic spread. *Cancer Gene Ther.* (2002) **9**(9):771-777.

71. SHIBATA MA, MORIMOTO J, OTSUKI Y: Suppression of murine mammary carcinoma growth and metastasis by HSVtk/GCV gene therapy using *in vivo* electroporation. *Cancer Gene Ther.* (2002) **9**(1):16-27.

72. LI S, ZHANG L, TORRERO M, CANNON M, BARRET R: Administration route- and immune cell activation-dependent tumor eradication by IL12 electrotransfer. *Mol. Ther.* (2005) **12**(5):942-949.

•• **Comparison of intratumoral electroporation with intramuscular electroporation.**

73. YAMASHITA Y, SHIMADA M, TANAKA S, OKAMAMOTO M, MIYAZAKI J, SUGIMACHI K: Electroporation-mediated TNF-related apoptosis-inducing ligand (TRAIL)/Apo2L gene therapy for hepatocellular carcinoma. *Hum. Gene Ther.* (2002) **13**(2):275-286.

74. IVANOV MA, LAMRIHI B, SZYF M, SCHERMAN D, BIGEY P: Enhanced antitumor activity of a combination of MBD2-antisense electrotransfer gene therapy and bleomycin electrochemotherapy. *J. Gene Med.* (2003) **5**(10):893-899.

75. LI S, XIA X, ZHANG X, SUEN J: Regression of tumors by IFN-α electroporation gene therapy and analysis of the responsible genes by cDNA array. *Gene Ther.* (2002) **9**(6):390-397.

76. KISHIDA T, ASADA H, SATOH E *et al.*: *In vivo* electroporation-mediated transfer of interleukin-12 and interleukin-18 genes induces significant antitumor effects against melanoma in mice. *Gene Ther.* (2001) **8**(16):1234-1240.

77. TSUJIE M, ISAKA Y, NAKAMURA H, IMAI E, HORI M: Electroporation-mediated gene transfer that targets glomeruli. *J. Am. Soc. Nephrol.* (2001) **12**(5):949-954.

• **First demonstration of direct delivery to kidneys.**

78. ISAKA Y, NAKAMURA H, MIZUI M *et al.*: DNAzyme for TGF-β suppressed extracellular matrix accumulation in experimental glomerulonephritis. *Kidney Int.* (2004) **66**(2):586-590.

79. TAKABATAKE Y, ISAKA Y, MIZUI M *et al.*: Exploring RNA interference as a therapeutic strategy for renal disease. *Gene Ther.* (2005) **12**(12):965-973.

•• **Demonstration of siRNA therapy coupled with electroporation.**

80. MIZUI M, ISAKA Y, TAKABATAKE Y *et al.*: Transcription factor Ets-1 is essential for mesangial matrix remodeling. *Kidney Int.* (2006) **70**(2):298-305.

81. YAZAWA K, ISAKA Y, TAKAHARA S *et al.*: Direct transfer of hepatocyte growth factor gene into kidney suppresses cyclosporin A nephrotoxicity in rats. *Nephrol. Dial. Transplant.* (2004) **19**(4):812-816.

82. NAKAMURA H, ISAKA Y, TSUJIE M *et al.*: Introduction of DNA enzyme for Egr-1 into tubulointerstitial fibroblasts by electroporation reduced interstitial -smooth muscle actin expression and fibrosis in unilateral ureteral obstruction (UUO) rats. *Gene Ther.* (2002) **9**(8):495-502.

83. TSUJIE M, ISAKA Y, ANDO Y *et al.*: Gene transfer targeting interstitial fibroblasts by the artificial viral envelope-type hemagglutinating virus of Japan liposome method. *Kidney Int.* (2000) **57**(5):1973-1980.

84. FUJII N, ISAKA Y, TAKABATAKE Y *et al.*: Targeting of interstitial cells using a simple gene-transfer strategy. *Nephrol. Dial. Transplant.* (2006) **21**(10):2745-2753.

85. FAVRE D, PROVOST N, BLOUIN V *et al.*: Immediate and long-term safety of recombinant adeno-associated virus injection into the nonhuman primate muscle. *Mol. Ther.* (2001) 4(6):559-566.

86. NATHWANI AC, DAVIDOFF AM, HANAWA H *et al.*: Sustained high-level expression of human factor IX (hFIX) after liver-targeted delivery of recombinant adeno-associated virus encoding the hFIX gene in rhesus macaques. *Blood* (2002) **100**(5):1662-1669.

87. REYNOLDS PN, HOLMES MD, ADACHI Y, KALIBEROVA L, CURIEL DT: A novel system for mitigation of ectopic transgene expression induced by adenoviral vectors. *Gene Ther.* (2001) **8**(16):1271-1275.

88. THOMA C, WIELAND S, MORADPOUR D *et al.*: Ligand-mediated retargeting of recombinant adenovirus for gene transfer *in vivo*. *Gene Ther.* (2000) 7(12):1039-1045.

89. LIU F, HUANG L: Improving plasmid DNA-mediated liver gene transfer by prolonging its retention in the hepatic vasculature. *J. Gene Med.* (2001) **3**(6):569-576.

90. LIU F, SONG Y, LIU D: Hydrodynamics-based transfection in animals by systemic administration of plasmid DNA. *Gene Ther.* (1999) **6**(7):1258-1266.

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

92. JAROSZESKI MJ, COPPOLA D, POTTINGER C, BENSON K, GILBERT RA, HELLER R: Treatment of hepatocellular carcinoma in a rat model using electrochemotherapy. *Eur. J. Cancer* (2001) **37**(3):422-430.

93. HARADA N, SHIMADA M, OKANO S *et al.*: IL-12 gene therapy is an effective therapeutic strategy for hepatocellular carcinoma in immunosuppressed mice. *J. Immunol.* (2004) **173**(11):6635-6644.

94. MURAMATSU T, ITO N, TAMAOKI N, ODA H, PARK HM: *In vivo* gene electroporation confers nutritionally-regulated foreign gene expression in the liver. *Int. J. Mol. Med.* (2001) 7(1):61-66.

95. SAKAI M, NISHIKAWA M, THANAKETPAISARN O, YAMASHITA F, HASHIDA M: Hepatocyte-targeted gene transfer by combination of vascularly delivered plasmid DNA and *in vivo* electroporation. *Gene Ther.* (2005) **12**(7):607-616.

96. PENG J, ZHAO Y, MAI J *et al.*: Inhibition of hepatitis B virus replication by various RNAi constructs and their pharmacodynamic properties. *J. Gen. Virol.* (2005) **86**(Part 12):3227-3234.

97. ZHANG L, NOLAN E, KREITSCHITZ S, RABUSSAY DP: Enhanced delivery of naked DNA to the skin by non-invasive *in vivo* electroporation. *Biochim. Biophys. Acta* (2002) **1572**(1):1-9.

98. DRABICK JJ, GLASSPOOL-MALONE J, KING A, MALONE RW: Cutaneous transfection and immune responses to intradermal nucleic acid vaccination are significantly enhanced by *in vivo* electropermeabilization. *Mol. Ther.* (2001) **3**(2):249-255.

99. GLASSPOOL-MALONE J, SOMIARI S, DRABICK JJ, MALONE RW: Efficient nonviral cutaneous transfection. *Mol. Ther.* (2000) **2**(2):140-146.

100. 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**(24):1780-1785.

101. MEDI BM, HOSELTON S, MAREPALLI RB, SINGH J: Skin targeted DNA vaccine delivery using electroporation in rabbits. I: efficacy. *Int. J. Pharmaceut.* (2005) **294**(1-2):53-63.

102. ISHIKAWA H, TAKANO M, MATSUMOTO N *et al.*: Effect of GDNF gene transfer into axotomized retinal ganglion cells using *in vivo* electroporation with a contact lens-type electrode. *Gene Ther.* (2005) **12**(4):289-298.

103. MATSUDA T, CEPKO CL: Controlled expression of transgenes introduced by *in vivo* electroporation. *Proc. Natl. Acad. Sci. USA* (2007) **104**(3):1027-1032.

•• **Employment of the Cre/loxP system in electroporation-mediated transfection.**

104. MO X, YOKOYAMA A, OSHITARI T *et al.*: Rescue of axotomized retinal ganglion cells by BDNF gene electroporation in adult rats. *Invest. Ophthalmol. Vis. Sci.* (2002) **43**(7):2401-2405.

105. DEZAWA M, TAKANO M, NEGISHI H, MO X, OSHITARI T, SAWADA H: Gene transfer into retinal ganglion cells by *in vivo* electroporation: a new approach. *Micron* (2002) **33**(1):1-6.

106. GAZDHAR A, FACHINGER P, VAN LEER C *et al.*: Gene transfer of hepatocyte growth factor by electroporation reduces bleomycin-induced lung fibrosis. *Am. J. Physiol.* (2007) **292**(2):L529-L536.

107. SEIDLER RW, ALLGAUER S, AILINGER S *et al.*: *In vivo* human MCP-1 transfection in porcine arteries by intravascular electroporation. *Pharm. Res.* (2005) **22**(10):1685-1691.

108. OTANI M, YOSHIDA M, IWASHITA H *et al.*: Electroporation-mediated muscarinic M3 receptor gene transfer into rat urinary bladder. *Int. J. Urol.* (2004) **11**(11):1001-1008.

109. COLOMBO MP, TRINCHIERI G: Interleukin-12 in anti-tumor immunity and immunotherapy. *Cytokine Growth Factor Rev.* (2002) **13**(2):155-168.

110. RAKHMILEVICH AL, TIMMINS JG, JANSSEN K, POHLMANN EL, SHEEHY MJ, YANG NS: Gene gun-mediated IL-12 gene therapy induces antitumor effects in the absence of toxicity: a direct comparison with systemic IL-12 protein therapy. *J. Immunother.* (1999) **22**:135-144.

111. HEINZERLING L, BURG G, DUMMER R *et al.*: Intratumoral injection of DNA encoding human interleukin 12 into patients with metastatic melanoma: clinical efficacy. *Hum. Gene Ther.* (2005) **16**(1):35-48.

112. HELLER L, MERKLER K, WESTOVER J *et al.*: Evaluation of toxicity following electrically mediated interleukin-12 gene delivery in a B16 mouse melanoma model. *Clin. Cancer Res.* (2006) **12**(10):3177-3183.

113. YEN HH, SCHEERLINCK JP: Co-delivery of plasmid-encoded cytokines modulates the immune response to a DNA vaccine delivered by *in vivo* electroporation. *Vaccine* (2007) **25**(14):2575-2582.

114. ISAKA Y, YAMADA K, TAKABATAKE Y *et al.*: Electroporation-mediated HGF gene transfection protected the kidney against graft injury. *Gene Ther.* (2005) **12**(10):815-820.

•• **Application of electroporation to transplantation.**

115. KOBAYASHI S, DONO K, TAKAHARA S *et al.*: Electroporation-mediated *ex vivo* gene transfer into graft not requiring injection pressure in orthotopic liver transplantation. *J. Gene Med.* (2003) **5**(6):510-517.

116. KOBAYASHI S, DONO K, TANAKA T *et al.*: Gene transfer into the liver by plasmid injection into the portal vein combined with electroporation. *J. Gene Med.* (2003) **5**(3):201-208.

117. PALMER TD, ROSMAN GJ, OSBORNE WR, MILLER AD: Genetically modified skin fibroblasts persist long after transplantation but gradually inactivate introduced genes. *Proc. Natl. Acad. Sci. USA* (1991) **88**(4):1330-1334.

118. YAMAMOTO S, YAMANO T, TANAKA M *et al.*: A novel combination of suicide gene therapy and histone deacetylase inhibitor for treatment of malignant melanoma. *Cancer Gene Ther.* (2003) **10**(3):179-186.

• **Discusses suicide gene therapy combined with histone deacetylase inhibitors.**

Website

201. http://www.wiley.co.uk/genmed/clinical/ The Journal of Gene Medicine Clinical Trial site (Accessed 10th August 2007).

Affiliation

Yoshitaka Isaka[†1] MD PhD &
Enyu Imai[2] MD PhD
[†]Author for correspondence
[1]Associate Professor,
Osaka University Graduate School of Medicine,
Divisions of Advanced Technology for Transplantation and Nephrology,
Suita, Osaka 565-0871, Japan
Tel: +81 6 6879 3746; Fax: +81 6 6879 3749;
E-mail: isaka@att.med.osaka-u.ac.jp
[2]Associate Professor,
Osaka University Graduate School of Medicine,
Division of Nephrology,
Suita, Osaka 565-0871, Japan