We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 6

DNA Electrotransfer: An Effective Tool for Gene Therapy

Aurore Burgain-Chain and Daniel Scherman

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/52528

1. Introduction

The concept of gene therapy was first introduced in the mid-80s, and is based on the delivery of genetic material (DNA or RNA) in the nucleus of patient cells, so that it is expressed and produces a therapeutic effect.

Different approaches can be considered:

- Correcting defective function by supplying a functional gene to the cells, thereby directly addressing the cause of a genetic disease.
- Transferring a gene encoding a therapeutic protein, in order to treat, prevent or slow the progression of certain diseases.
- Introducing a gene leading to the death of a diseased cell
- Introducing antisense DNA inhibiting the formation of a protein or the replication of a virus

Originally developed for monogenic diseases, and therefore associated with the compensation of genes whose alteration is responsible for diseases, the concept of gene therapy has rapidly expanded to the use of DNA as a new type of drug. Therefore, gene therapy leads to indications which are far beyond the case of genetic diseases, since a DNA drug can, in principle, replace any medication which will control protein synthesis. Gene therapy seems an alternative choice to fight against diseases currently treated imperfectly, or not treated with conventional pharmaceutical approaches.

In addition, gene therapy has many advantages compared to the administration of recombinant proteins. Indeed, recombinant proteins are costly and their elimination from the blood

flow is fast, while gene therapy leads to a long-term and potentially regulated production of a therapeutic protein. Gene therapy also allows the localized expression of the transgene, avoiding any risk associated with the presence of a systemic exogenous protein.

The main limitation of current gene therapy is the development of effective gene transfer. Indeed, in order to reach the cell nucleus, the therapeutic gene has to cross several biological barriers. Therefore, the success of any gene therapy requires the development of efficient and appropriate methods and vectors for introducing the gene of interest into target cells. The ideal vehicle for gene transfer must have the following properties: (1) specificity to target cells, (2) localized gene delivery, (3) resistance to metabolic degradation and/or attack by the immune system, (4) minimum side effects, and (5) eventually controlled temporal transgene expression [1].

Many methods of *in vivo* gene transfer exist and are generally classified into two main categories: viral and non viral. Viruses are very efficient vehicles for gene transfer; however their use is limited by high production costs and safety concerns, such as immune response, possible pathogen reversion, mutagenesis and carcinogenesis. Considering these limitations, the delivery of therapeutic genes to target cells by non viral approaches may be of great value for the development of gene therapy. Among these approaches, *in vivo* electroporation, also called *in vivo* electropermeabilization or *in vivo* electrotransfer, has proven to be one of the simplest and most efficient methods for gene therapy, while at the same time being safe, cheap, and easy to perform.

In vivo electrotransfer is a recent physical technique for gene delivery in various tissues and organs, which relies on the combination of plasmid injection and delivery of short and defined electric pulses. This process results in the association between cell permeabilization and DNA electrophoresis. Skeletal muscle have now been frequently electrotransfered, since it offers promising treatment for muscle disorders, but also a way for systemic secretion of therapeutic proteins, by converting skeletal muscles into an endocrine organ: the protein produced can diffuse into the vascular system and circulate throughout the body to exert a physiological and potentially therapeutic effect. Many published studies have demonstrated that plasmid electrotransfer can lead to long-lasting therapeutic effects in various pathologies such as cancer, rheumatoid arthritis, muscle and blood disorders, cardiac diseases, etc... Indeed, the physical method of electrotransfer allows for greater efficiency of gene transfer after a single injection and improves protein expression by several orders of magnitude, as compared to DNA injected in the absence of electrotransfer. Therefore, plasmid electrotransfer can be considered a powerful tool for gene therapy.

The scope of this chapter encompasses the methods of electrotransfer, its implementation, mechanism, optimization and therapeutic applications.

2. Description of the electrotransfer technique

In 1982, E. Neumann and his collaborators demonstrated *in vitro* the possibility of introducing DNA into cells using electrical pulses [2]. These electric pulses would cause the destabi-

http://dx.doi.org/10.5772/52528

lization and permeabilization of the plasma membrane of suspended cells, thus promoting the entry of exogenous DNA into these cells. Two years later [3], the confirmation of this result opened the way for the development of electroporation (or electropermeabilization) into bacterial [4], fungal [5] vegetal or animal cells. This method is routinely used now. The optimization of electrical parameters is critical to allow transient permeabilization, together with a satisfactory cell survival rate [6].

In initial studies, *in vivo* DNA electrotransfer has been tested in the skin in 1991, by the use of exponentially decaying electrical pulses, and in 1996 in the liver using trains of short 100 μs pulses [7]. In 1998, four independent teams showed the effectiveness of electrotransfer using pulses of long duration (5-50ms): in skeletal muscle, our team in collaboration with that of Luis Mir [8] and Aihara [9], in tumors, Rols *et al.* [10] and in liver Suzuki *et al.* [11]. *In vivo* DNA electrotransfer has now been successfully used in a broad range of target tissues and organs including for example : arteries [12], skin [13], tendon [14], bladder [15], cornea [16], the retinal cells [17], spinal cord [18]and brain [19].

Electropermeabilization can also be used to deliver chemical drugs into the cells: e.g. electrochemotherapy in tumors, with the use of bleomycin, developed since 1991 [20]. Several clinical trials are underway [21], primarily for the treatment of subcutaneous or skin tumors [22, 23] and recently for the treatment of breast cancer with cisplatin [24] (For a review see [25]).

3. Mechanism of electrotransfer at the cell level

The technique of electroporation for the transfer of nucleic acids has been used since the 80s, however its exact mechanism is not yet completely elucidated [26, 27]. At the cell level, it seems that two phenomena occur: first the permeabilization of the cell to small molecules, probably due to a destabilization of the cell membrane, and secondly the transport of DNA by electrophoresis.

3.1. Permeabilization

The lipid bi-layer of the plasma membrane separates two solutions with very high ionic conductivity: the cytoplasm and the extracellular medium. Typically, at rest, the membrane potential difference (ΔVm_0) is around -70mV. When an electric field is applied to a cell, the resulting current induces an accumulation of electric charges at the cell membrane which leads to a variation of thistransmembrane potential. And if the transmembrane potential exceeds a certain threshold value, the cell membrane is disorganized and structural changes occur. That is a necessary condition for an effective gene transfer [28].

Shall the cell be considered a hollow sphere where the thickness of the membrane is negligible vis-à-vis the cell radius, then the transmembrane potential difference ΔVm induced by an electric field is, as described by Schwann's equation:

$$\Delta Vm = f.g.r.E.cosq.(1-exp(-t/t)) \quad (1)$$

Thus, the transmembrane potential difference ΔVm is proportional to

- the cell radius (r)
- the magnitude of the electric field (E) (expressed in volts/cm)
- the cosine of (θ), its incidence angle,
- a cell shape factor (f)
- the conductivity of the medium (g)
- the pulse duration for which the electric field is applied (t)
- the charging time constant of the cell (τ).

If the membrane is seen as a pure dielectric object, g is equal to 1. Under the conditions used for cellular electroporation, the pulse duration is significantly longer (of a few hundred microseconds to a few milliseconds) than the charging time constant of the cell, which is of the order of a few microseconds. The equation can be simplified to:

$$\Delta Vm = f.\ r.E.cosq \quad (2)$$

This transmembrane potential difference ΔVm is not uniform on the surface of the cell: the induced transmembrane potential is maximal at the points of the cell facing the electrodes (θ = 0 and π).

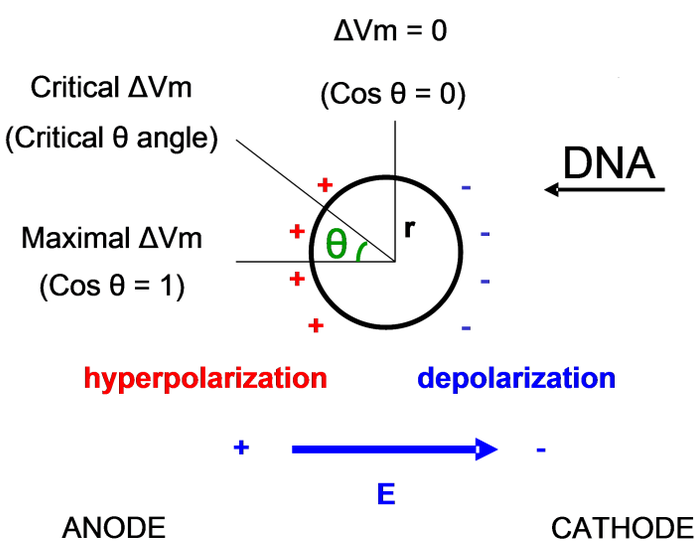

Figure 1. Theoretical model of the cell for electroporation: E, the electrical potential induces ΔVm, a transmembrane potential difference which dependent on r, the radius of the cell and θ, the angle between the direction of electric field and the normal to the tangent of the membrane of the cell at this point

The membrane is off-balance and becomes transiently permeable when the sum: ΔVm_0 (at rest) + ΔVm (induced) reaches a threshold value of about 200mV [29]. Thus, the greater the difference between the threshold value and the value applied, the greater the surface area is permeabilized. However for a given electric field, beyond a certain angle, the ΔVm falls below the threshold value of permeabilization. The relationship between the applied electric field and the permeabilized surface was demonstrated by *in vitro* fluorescent labeling of permeabilized areas of the cell [30]. Moreover, these studies have shown that it is the face of the cell toward the anode side which is permeabilized first, the negative potential of the cell being in addition to that induced by the external electric field.

One theory suggests that the DNA enters into the cell through pores which are generated by electrical stimulation [32]. The electropermeabilization creates relatively stable "electropores" [2, 33]. But these pores have never been visualized. The plasmid DNA may optionally pass the membrane after a step of binding to the surface of the cell and by diffusion.

The second phenomenon necessary for gene transfer by electroporation is the electrophoresis of negatively charged DNA.

3.2. DNA electrophoresis

The occurrence of an electrophoretic process has been demonstrated *in vitro* [31]. Various studies have shown this electrophoretic effect: Klenchin *et al.* demonstrated that DNA has to be present at the time of the pulses [31]. Furthermore, they showed that the transfection efficiency depends on the polarity of the electric field. Sukharev *et al.* also showed *in vitro* that short pulses of high voltage (HV) induce membrane permeabilization but not transfection, whereas long pulses at low voltage (LV) do not induce permeabilization or transfection. However, the sequence “high voltage pulses followed by low voltage pulses” provides a transfection. An hypothesis is proposed that transfection of cells permeabilized by high voltage is only possible if low voltage pulses can subsequently mediate DNA electrophoresis [34].

The role of permeabilization and electrophoresis was demonstrated directly at the cell level by fluorescence microscopy [35]. This work shows that interaction between the membrane and electropermeabilized DNA is induced in response to electrical pulses of a few milliseconds. DNA electrophoretically accumulates on the cathode side of the cell without immediately moving into the cytosol (Figure 1). Thus DNA must be present during the pulse and electrophoresis induced by the electric field promotes its transfer through the membrane, but it is only during the following minute that DNA crosses the electropermeabilized membrane [36]. There is a direct relationship between the DNA/membrane interaction and transfection efficiency: the larger the contact surface between DNA and the membrane, the higher is the expression [27].

4. Mechanism of *in vivo* electrotransfer

In the early 90’s, the first studies about *in vivo* electroporation appeared. They primarily concerned the transfer of chemical molecules. The first real demonstration of *in vivo* cellular

electropermeabilization was performed on tumors after injection of bleomycin, a cytotoxic anticancer agent, [22, 37]. The effectiveness of bleomycin depends on its intracellular concentration, but this drug penetrates poorly into cells. Therefore, a better penetration of bleomycin was measured after application of electric pulses to tumors, leading to an enhanced desired cytotoxicity.

Most studies are pointing to a mechanism of *in vivo* electrotransfer comparable to the mechanism of *in vitro* electrotransfer described above,which can be extended to the whole tissue: several steps have to take place, including cell permeabilization beyond a threshold value of local electric field. In 1999, we evaluated on one hand cell permeabilization following the application of electrical pulses by measuring the ability of muscle cells to capture a small radioactive hydrophilic molecule complexe of EDTA Chelating 51 chromium (^{51}Cr-EDTA), and on the other hand, transgene expression for evidence of DNA entry [38, 39]. The uptake of ^{51}Cr-EDTA was similar whether injected thirty seconds before or after applying electrical pulses. In contrast, DNA injected after the electrical impulses does not penetrate into cells. This suggests that DNA must be present *in situ* at the time of electrical pulses to obtain an efficient cell transfection, and that there is a direct, active effect of the electric field on the DNA molecules to promote their entry into cells. Hence the current mechanistic hypothesis of gene electrotransfer necessitates not only a permeabilization of cell membranes but also a DNA electrophoresis.

This hypothesis is supported by the study of Bureau *et al.* [40] of gene electrotransfer in skeletal muscle of mice with different combinations of long pulses of low voltage (LV, i.e. electrophoretic pulses) and short pulses of high voltage (HV, i.e. permeabilizing pulses). Only the combination of a HV-pulse followed by a LV-pulse provided efficient gene transfer. Further studies confirmed that HV-pulses are related to permeabilization, while LV-pulses are related to the efficiency of DNA electrophoresis [41]. The importance of cell permeabilization was also studied by magnetic resonance imaging using a gadolinium complex as contrast agent (dimeglumine gadopentate): the zone of di meglumine gadopentate complex permeabilization is identical to the area expression of electrotransfered DNA [42].

The destabilization of cell membranes and the electrophoretic effect are probably not the only mechanisms involved in gene transfer by electroporation. Scientists have discussed the importance of energy metabolism (ATP and ADP) for the passage of DNA through the permeabilized membrane and its migration to the nucleus [28].

Other studies suggest a mechanism of DNA transport by endocytosis [43]. These same studies show that transfection efficiency does not decrease if the electrical pulses are delivered up to four hours after injection of DNA, while other studies show that most of the injected DNA is degraded in first hours after injection [44]. We also confirmed that after an intramuscular injection, most of the DNA is degraded and eliminated quickly. However, a small proportion of DNA is preserved and provides a source of stable DNA which can been electrotransfered [45].

In summary, the molecular mechanism of *in vivo* DNA electrotransfer is still under investigation. It likely corresponds to multiple steps whose elucidation and understanding of re-

spective contribution could help to develop more effective electrotransfer strategies and protocols.

5. Electrotransfer into practice

The *in vivo* electrotransfer technique is particularly easy to implement: a solution of plasmid DNA (i. e. a circular nucleic acid) in isotonic saline (NaCl, 150mM) is injected into the target tissue with a syringe, and electric pulses are then delivered by means of electrodes placed on either side of the injection site and connected to a generator (Figure 2). Electrodes can be either needles or plates.

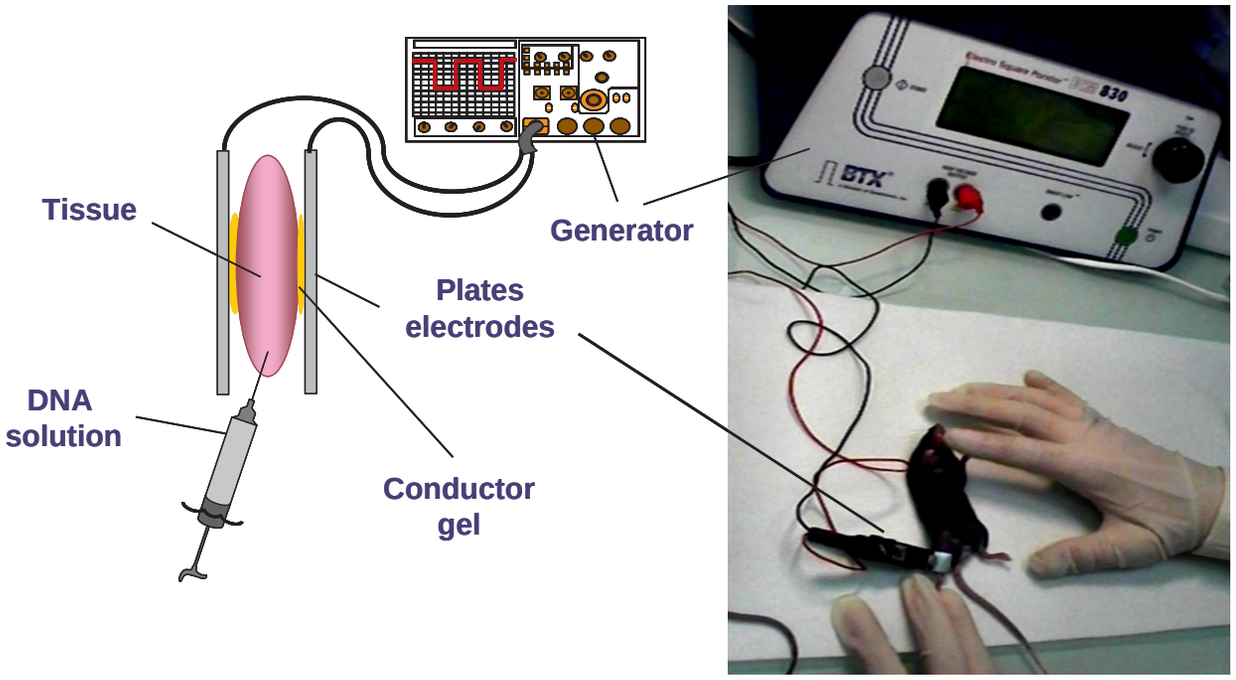

Figure 2. Experimental set up for intramuscular plasmid electrotransfer in mice

This technique allows a site specific gene transfer. It is relatively efficient in skeletal muscle and is applicable to many other tissues such as brain, liver, skin, bladder, kidney, lung, cornea, retina, testis, tumor tissue etc... for more details see [46]. Electrotransfer can also be used in a wealth of animal models, ranging from rats and mice to sheeps [47] and cows [48] and even fish [49].

5.1. Operating parameters

The efficiency of gene transfer depends on the target tissue, the delivered DNA and electric pulses parameters. The aim is to deliver, into each tissue, electrical pulses that can cause the permeabilization of cell membranes and DNA transfer, while remaining below the toxic threshold. Otherwise, local cell death by necrosis of the treated cells would occur, followed

by tissue regeneration, which would induce the loss of the benefit of the treatment (but with no toxicity at the level of the whole organism). Therefore, optimal conditions for the DNA electrotransfer in a targeted tissue result from a compromise between the efficiency of DNA transfer and minimal cellular toxicity.

5.1.1. The electrodes

The choice of electrodes depends on the target tissue and the size of the treated animal. It is critically important and should be carefully considered. For an electrotransfer on a small animal in a tissue such as skeletal muscle, or liver tumor, most experimenters use electrodes made of two plates attached to a clamp (Figure 3, left). Indeed, this type of electrodes can be easily applied externally on each side of the interested tissue. Because one key parameter is the electric field, which is related to the ratio between the voltage applied and the distance between electrodes, this latter distance should not be too large in order to avoid prohibitive high voltage *in vivo* delivery. Thus, for animals of larger size, needle electrodes (Figure 3, right) are more often used than external plates.

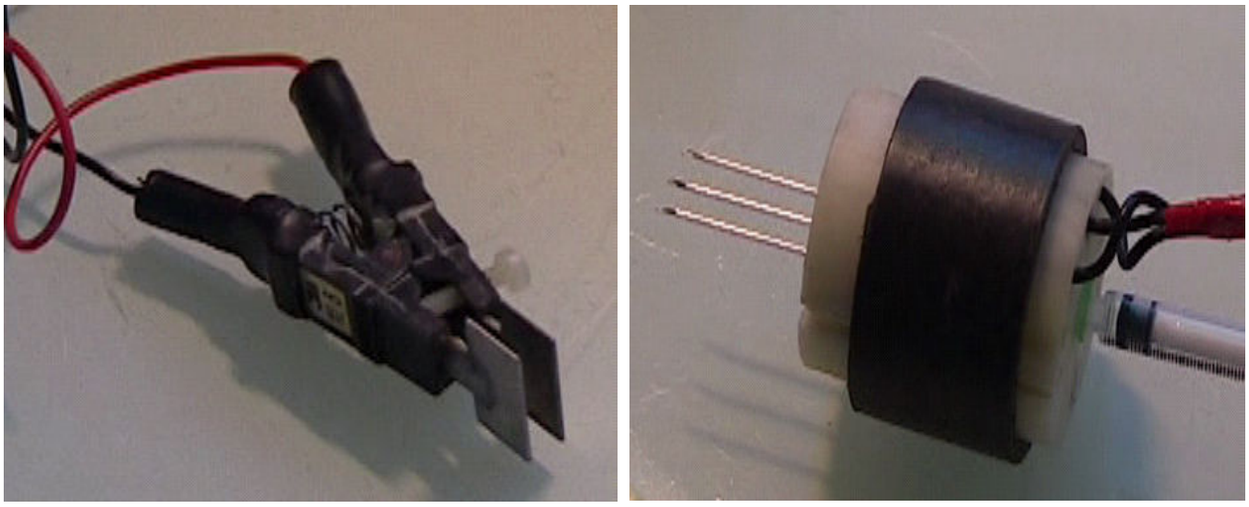

Figure 3. Examples of electrode plates for external use (left) and needle electrodes for internal use, designed by the company Sphergen (right).

5.1.2. Electrical parameters

Knowing the magnitude and distribution of electric field is very important for both efficient gene transfer and reduced toxicity. The distribution of the electric field is dependent on both the tissue and the type of electrodes, which causes variations in the effective magnitude of the field in the tissue area of interest. The electric field distribution is more homogeneous when using plate electrodes than with needle electrodes, and for a given setting, the resulting electric field is lower with needle electrodes that with electrode plates [38].

Moreover, it is necessary to determine, for each tissue and each species, the threshold values of the electric field magnitude, i.e. the permeabilization threshold (reversible) and the cell

damage threshold (irreversible), in order to define optimal electrical conditions for gene transfer with minimal toxic effects. Micklavic *et al.* have developed a system combining numerical predictions and experimental observations in order to determine these thresholds in the case of needle electrodes used in rat liver for drug delivery [50].

Different types of electrical pulses can be applied: unipolar square pulses, bipolar square pulses, or pulses with exponential decay [51]. The exponentially decaying pulses, colloquially referred to as "exponential pulses" are mainly used *in vitro* with a time constant dependent on the resistance of the incubation media. The square pulses are preferred *in vivo*, since the voltage and pulse duration can be set independently of the electrical resistance of the targeted tissue. Unipolar square pulses are the most widely used for electrotransfer experiments, while bipolar squares pulses are rather used for electrophysiology [52].

5.2. Toxicity

Tissue damage can be caused by electrotransfer and thus limits the efficiency of transfection [53]. The cell permeabilization is the main toxicity factor: it leads to an inward diffusion of the external medium as well as leakage of intracellular content, thus changing the composition of the latter. This toxicity can be reduced by minimizing the duration and the extent of permeabilization.

Other factors of toxicity have been described such as oxidative stress due to the formation of free radicals near the electropermeabilized membrane [6, 54]. It was also shown that electrotransfer induced muscle damage dependent on the amount of DNA injected [55]; these lesions disappear within two months after injection.

In our laboratory, histological analyzes of muscle slices have shown that the application of electric fields optimized for gene transfer does not induce gene expression markers of stress and cellular toxicity [56]. Other experiments have allowed to conclude that, even in optimized conditions, very little muscle damage is generated: few inflammatory lesions are observed with a maximum in the first seven days after the electrotransfer, but these disappear rapidly in less than three weeks [57-58].

It is also possible to reduce the extent of damage by increasing the accessibility of DNA to target cells. Indeed, studies have shown that improving the plasmid distribution leads to an increase in transgene expression. Thus, the value of the electric field used can be reduced. Better distribution can be obtained for example by pre-injection of hyaluronidase [59], an enzyme that degrades hyaluronic acid, which is a major component of the extracellular matrix [60]. This pretreatment allows for the same expression level, using lower voltages while reducing muscle damage [61]. A pre-injection of sucrose may also improve the distribution of DNA, by creating spaces between the muscle fibers [62]. Similarly, a pre-injection of poly-L-glutamate, an anionic polymer, seems to increase the internalization of the plasmid inside the cell and/or to reduce its degradation [63], and therefore increases the expression of exogenous gene.

5.3. Target tissues

During recent years, electrotransfer has been applied in various animal species to many tissues, including skeletal muscle, skin, liver, lungs, kidneys, joints, brain, retina, cornea, etc... [64]. The optimal parameters of a given electrotransfer should be determined based on the cell type and species, since these parameters strongly depend on tissue organization and the size of the transfected cells.

5.3.1. Skeletal muscle

One of the most widely used tissues for electrotransfer is skeletal muscle. The DNA electrotransfer into skeletal muscle was discovered independently by three teams [8, 9, 52]. Indeed, skeletal muscle offers many advantages:

- a large, easy access;
- sets of muscle fibers are parallel to each other: many fibers might have an optimal orientation relative to the electric field, which promotes even transfer across the entire length of the fibers;
- unlike other cells, muscle cells have multiple nuclei flattened against the cell membrane, which facilitates DNA trafficking to the nucleus;
- muscle fibers do not divide, ensuring long-term gene expression, notwithstanding the absence of regeneration due to injury or cytotoxic immune response;
- finally, a major advantage of skeletal muscle lies in its ability to produce and release biologically active proteins into the bloodstream, due to the strong vascularisation.

Combined together, these features can turn muscle into systemic drug delivery system for distant targets [65]. Interestingly, the cotransfection of multiple unlinked genes can be easily performed by electroporation [66]. For examples of electrotransfer in skeletal muscle in various mammalian species see [46].

5.3.2. The skin

The skin is, as muscle, also a widely used tissue for DNA electrotransfer, mostly because:

- this tissue is easily accessible and a large area of tissue can be treated;
- keratinocytes, which are epidermal cells, can synthesize and secrete therapeutic proteins that reach the bloodstream;
- by its natural function of a biological barrier, the skin contains cells that present antigens and is therefore an organ of choice for applications in DNA vaccination;
- the epidermal cells have a short lifespan, which can be useful for treatments requiring a brief period of expression.

However, skin structure [67] does not facilitate gene transfer. In particular, the top layer (stratum corneum or horny layer) is a major barrier [68, 69]. But a high level of expression in

the skin from a single injection could still be observed [70, 71]. Moreover Dujardin *et al*. have shown that square or exponential pulses induce moderate and reversible effects on the skin without inflammation or necrosis, while transiently permeabilizing the skin and thus allowing the passage of molecules [72].

5.4. Optimization of *in vivo* electrotransfer conditions

An important goal for gene transfer applications is the level and duration of gene expression. To determine optimal conditions which maximize efficiency while reducing tissue damage, different protocols have been used to improve the access of plasmids to targeted cells. As already described, improved plasmid distribution in the skeletal muscle leads to an increase in DNA expression. Accordingly, Cemazar *et al.* showed recently enhanced transfection efficiency of gene transfer by pretreatment of tumors with hyaluronidase and/or collagenase, two enzymes which modulate components of the extracellular matrix [73].

A secretion signal can be also added to the transgene sequence : we have recently shown that by modifying the cellular localization of the produced protein by adding a secretory signal, the production and secretion of this protein is enhanced, thus enhancing biological effect [74].

We have also shown that codon optimization of the transgene (i.e. retaining the natural amino acid sequence but using the preferred host animal codons) leads to increase in the expression of the protein of interest [74].

Another method to increase the stability of the protein produced in the blood circulation is to increase its size in order to avoid kidney excretion. Thus, the construction of fusion proteins, for instance by fusing a therapeutic protein with an IgG constant [75], appears a simple way to deliver enhanced levels of secreted proteins without altering their biological activities.

The enhanced protein expression, and so their biological effects, also depends of the injection regimen and the administered plasmid dose [74].

6. Applications of plasmid electrotransfer

DNA electrotransfer is a recent technique of has not yet successfully completed all stages of clinical development, but this is progressing. The first Phase I human clinical trial has been initiated in U.S. by the company Inovio Biomedicals, for the treatment of skin cancer [76]. Since then, the delivery of plasmid DNA encoding therapeutic genes has been tested extensively in preclinical melanoma models [77].

Applications designated as "therapeutic" which are mainly reported in the literature have been demonstrated on animal models of human diseases. The main potential therapeutic areas cover cancer [78], cardiovascular diseases [75], autoimmune diseases [79], monogenic diseases [80], organ-specific disorders [81] and vaccination [82, 83]. Different examples show

the efficiency of plasmid electrotransfer to produce therapeutic proteins in various pathologies [46]: all these experiments showed an improvement in symptoms of the relative disorder.

6.1. Cancer

Cancer accounts for major field of application trials of gene therapy. Different strategies can be broadly grouped into four main categories:

a. Stimulation of the immune response against a tumor [84],

b. Use of suicide genes [85-87];

c. Repair cell cycle defects caused by the loss of tumor suppressor genes or oncogene activation [88],

d. Inhibition of tumor angiogenesis [89].

These strategies can be combined to obtain synergistic results. For example, a combination of HSV-TK-suicide gene therapy and IL-21 immune gene therapy byelectrotransfer improves antitumor responses in mice [90]. Moreover, *in vivo* electrotransfer could be used in combination with other strategies such as chemotherapy, because these two approaches use different mechanisms to kill cancer cells, and thus a synergistic effect may be obtained.

Actually, electroporation of DNA encoding cytokines into tumors is extensively studied: IL-12 [91], IL-18 [92], IFN-α [93] have been shown to reduce tumor growth and increase survival times in different tumor models. Other interesting results are represented by the inhibition of tumor growth in various models with plasmids encoding metaloproteinase-3 inhibitor for the treatment of prostate cancers [94], or encoding endostatin for his therapeutic efficacy in mouse-transplanted tumors [95].

All these experiments show the potential of in vivo electrotranfer for cancer treatment. And the strategy used, i.e. the direct intra-tumoral plasmid electrotransfer, is well suited for the local production of therapeutic proteins. However, since the efficacy of gene transfer into tumor cells *in vivo* is generally low, intramuscular electrotransfer can also be efficiently used for distal tumor treatment. Indeed, an important application of the technique of plasmid electrotransfer is the protein secretion by skeletal muscle: the produced protein, such as, for instant, an immunostimulating cytokine, can diffuse into the vascular system and circulate throughout the body to exert a physiological effect, particularly therapeutic. This distal approach may be very powerful for surgically inaccessible tumors, such as head and neck tumors.

Finally, the intramuscular electrotransfer of a plasmid encoding the prostate membrane specific antigen (PMSA) has been tested in a human clinical trial of prostate cancer active immunotherapy [96]. DNA fusion-gene vaccination in patients with prostate cancer induces high-frequency CD8 (+) T-cell responses and increases PSA doubling time [97].

6.2. Monogenic diseases

Monogenic diseases with an identified defective gene have been the first diseases targeted by gene therapy approaches. Among these diseases, Duchenne muscular dystrophy (DMD), which is characterized by the absence of dystrophin, is a good model, since even a small amount of dystrophin would be sufficient to reverse the clinical phenotype of the disease. An approach to eventually restore this protein in patients with DMD is to introduce into their muscles a plasmid encoding dystrophinc DNA. Pichavant *et al.* were the first to demonstrate local restoration of full-length dog dystrophin in dystrophic dog muscle by DNA electrotransfer [98].

6.3. Hematopoietic factor deficiency

Erythropoietin (EPO) is another good candidate for gene therapy applications because a small amount will produce the desired physiological effect of raising the hematocrit. Numerous studies, in particular by our own group, report efficient EPO secretion after plasmid electrotransfer, with a therapeutic effect in anemia and beta thalassemia. The use of intramuscular plasmid electrotransfer for EPO gene delivery in mice increased approximately 10 to 100-fold the expression of this gene, as compared to naked DNA alone [99, 100]. Moreover with this method, the protein in circulation and hematocrit levels were stable for 2 to 6 months after a single injection of minimal amounts (as little as 1 μg) of a plasmid carrying the mouse EPO cDNA. Several studies also showed that EPO expression could be regulated, for instance by co-administering an EPO encoding plasmid under the control of a tetracycline-inducible promotor and a second plasmid carrying the reverse tetracycline-dependent transactivator protein [100, 101]. All these studies exemplified that plasmid DNA electrotransfer can efficiently produce enough amounts of transgenic EPO in normal mice.

In collaboration with the group of Y. Beuzard, we have demonstrated the relevance of intramuscular electroporation of an EPO-expressing plasmid in a mouse model of human β-thalassemia, a severe genetic disease, leading to a durable and dose-dependent phenotypic correction of this severe genetic disease [102]. In addition, we have also shown that it is possible to produce fusion protein by plasmid DNA electrotransfer [103]: indeed since the bridging of two adjacent EPO receptors triggers a conformational change that initiates signal transduction [104], we have hypothesized that the fusion of two EPO molecules might lead an increase in intrinsic activity of EPO. Thus, we demonstrated that the injection of EPO dimer encoding plasmid by electrotransfer in a skeletal muscle of β-thalassemic mice induces an increase in the biologic specific activity of this EPO dimer in comparison with the activity of monomer [103].

Furthermore the secretion peak of therapeutic protein following DNA administration is potentially deleterious. We reported that muscular electrotransfer of low doses of plasmid can be repeated several times to weeks or even months after the initial injection, and that this strategy leads to efficient, long-lasting and non-toxic treatment of β-thalassemic mouse anemia avoiding the deleterious initial hematocrit peak and maintaining a normal hematocrit with small fluctuation [105].

In addition, Gothelf *et al.* demonstrate that gene electrotransfer to skin of even small amounts of EPO DNA can lead to systemically therapeutic levels of EPO protein [106].

6.4. Cardiovascular diseases

Gene therapy is an attractive strategy for the treatment of cardiovascular disease. However, using current methods, the induction of gene expression at therapeutic levels is often inefficient. Therefore DNA electrotransfer directly into heart may enhance the delivery of therapeutic protein as shown the team of R. Heller : the electroporation method ameliorates the delivery of a plasmid encoding an angiogenic growth factor (vascular endothelial growth factor, VEGF), which is a molecule previously documented to stimulate revascularization in coronary artery disease [107]. Ayuni *et al.* demonstrated that, unlike the usual methods to treat coronary artery diseases, electrotransfer applied directly into the beating heart enhances the delivery of a plasmid injected via the coronary veins after transient occlusion of the coronary sinus [108]. These results show that *in vivo* electroporation mediated gene transfer is feasible and safe,in particular to the heart. Finally, in skin, D. Dean reported that using electroporation in skin enhances delivery of plasmid DNA encoding fibroblast growth factor-2 (FGF-2) to induce neovascularization as atherapy for ischemia in a rat model [109].

6.5. Eye diseases

The eye is an isolated organ difficult to reach via systemic administration. Eye diseases are treated with intra- or periocular injections and these repeated injections bear the risk of adverse effects, mainly infections, and are poorly tolerated by the patients. The use of DNA electrotransfer technique is therefore possible to deliver a local treatment. Our team associated with an ophtalmology group has developed electrotransfer to the ciliary muscle, which is a particular smooth muscle with some characteristics of striated skeletal muscle, for the local treatment of inflammatory eye disease. This approach led to production and secretion of therapeutic levels of TNFα soluble receptor in the ocular media, and not in the serum, thus preventing clinical and histological signs in a rat uveitis model [110, 111]. Recently, suprachoroidal electrotransfer with a reporter plasmid to transfect the choroid and the retina without detaching the retina has been reported [112]. Not only choroidal cells but also RPE, and potentially photoreceptors, were efficiently transduced for at least a month, without ocular complications. This minimally invasive non-viral gene therapy method may open new prospects for human retinal therapies.

6.6. Obesity and diabetes

As mentioned above, skeletal muscle can be an efficient platform for the secretion of erythropoietin (EPO), which displays a variety of metabolic effects when it is expressed in supraphysiological levels. Hojman *et al.* have proposed to overexpress EPO in muscle by electrotransfer of plasmid in the aim to protect mice against diet-induced obesity and normalize glucose sensitivity, associated with a shift to increased fat metabolism in the muscles [113]. Similar results were obtained after DNA electrotransfer of plasmid encoding the carni-

tine palmitoyltransferase 1 (CPT1), the enzyme that controls the entry of long-chain fatty acyl CoA into mitochondria: an overexpression of CPT1 led to enhance rates of fatty acid beta-oxidation and improved insulin action in muscle in high-fat diet insulin-resistant rats [114]. In the same model, electrotransfer of the orphan nuclear receptor (Nur77) significantly ameliorates the effect of this protein on glucose metabolism [115].

6.7. Vaccination and passive immunization by antibody production

The prospect of inducing an immune response to a protein expressed *in vivo* directly from administered DNA vaccine represents an attractive alternative to other modes of vaccination. Plasmid electrotransfer has been used in genetic immunization to produce antigenic proteins. It is now well established that genetic immunization induces both durable cellular and humoral responses [116]. This type of immunization is often developed for vaccination (virus or antibacterial), for anticancer active immunotherapy, and also to induce in animals the production with high yield of antibodies against a given antigen.

Since electrotransfer efficiently transfers genes compared to a single injection of plasmid, improving antigenic protein expression by several orders of magnitude, the antibody titer and the quality of the immune response are also improved [117], with an increasing factor of 100 in mice after electrotransfer of a plasmid encoding a surface antigen of hepatitis B [118]. High titers of antibodies were also obtained in mice and rabbits after i.m. electrotransfer of a plasmid encoding an envelope glycoprotein of hepatitis C [119], and in mice after electrotransfer of a plasmid encoding a protein of Mycobacterium tuberculosis [120]. In the laboratory, it was shown that i.m. electrotransfer of a plasmid encoding the influenza hemagglutinin induces a better immune response in mice that a single i.m. injection [121]. And recently, we have assessed the potential of i.m. electrotransferin mouse to produce neutralizing antibodies, with high titer, against botulinum toxins, the most powerful poison in the world in present time [74]. We have optimized DNA electrotransfer for genetic immunization against botulinum antigen. This DNA immunization has been used in rabbits to induce antibodies production which is compatible with industrial development of antiserum production for a human therapeutic use (Burgain *et al.*, unpublished results).These examples show that it is possible to obtain high titers neutralizing antibodies in animals by DNA electrotransfer.

Monoclonal antibodies are increasingly being used in a wide range of clinical applications in the field of autoimmune disease, cancer and infectious disease. The production and secretion by electrotransfered muscle of monoclonal antibodies has been demonstrated by our group and the one of I. Mathiesen, independently [83, 122]. These studies demonstrated that the co-transfection of two naked plasmids encoding the heavy and light G immunoglobulin chains led to the secretion of fully assembled and functional immunoglobulin molecules. The successful neutralization of various pathogens resulted from monoclonal antibody secretion by electrotransfered muscle, raising the possibility of clinical passive immunization applications.

7. Conclusion

In vivo electrotransfer is a non-viral technique which has emerged as an efficient, user-friendly and cheap gene transfer method which issuited for a wide range of tissues and species. Moreover, *in vivo* electrotransfer can be used for either local or distal effect by secretion of the transgenic protein into the bloodstream. The skeletal muscle is able to produce functional proteins with adequate post-translational modifications, which means that the muscle can be used as an endocrine organ for the production of therapeutic secreted proteins targeting systemic diseases. It is now established that therapeutic levels of circulating proteins can be reached in animal models. And since DNA does not induce any immune response, plasmid electrotransfer can be repeated as often as desired (Scherman *et al.*, unpublished results).

The understanding of the precise mechanism of electrotransfer, the optimization of its realization, the improvement of plasmids and of the structure of the encoded protein will bring more efficiency and above all more safety to the method, should it be applied to humans. Several clinical trials have been conducted and/or are still in progress. For more details see http://www.clinicaltrials.gov/ct2/results?term=electroporation. These clinical trials are mainly conducted against infectious diseases such AIDS, hepatitis B, malaria, dengue, influenza... and various cancer types such as ovarian cancer or renal cancer, melanoma, cancers caused by human papillomavirus... Thus, DNA electrotransfer appears as a powerful and promising tool not only for gene therapy, but also for in vivo gene delivery at the laboratory level within the frame of physiological studies.

Author details

Aurore Burgain-Chain and Daniel Scherman

Unit of Chemical and Genetic Pharmacology and of Bioimaging, CNRS Paris, Université Paris Descartes; Chimie Paris Tech; Paris-Sorbonne PRES, France

References

[1] Mehier-Humbert S, Guy RH. Physical methods for gene transfer: improving the kinetics of gene delivery into cells. Adv Drug Deliv Rev. 2005 Apr 5;57(5):733-53.

[2] 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(7):841-5.

[3] Potter H, Weir L, Leder P. Enhancer-dependent expression of human kappa immunoglobulin genes introduced into mouse pre-B lymphocytes by electroporation. ProcNatlAcadSci U S A. 1984 Nov;81(22):7161-5.

[4] Calvin NM, Hanawalt PC. High-efficiency transformation of bacterial cells by electroporation. J Bacteriol. 1988 Jun;170(6):2796-801.

[5] Ganeva V, Galutzov B, Teissie J. Fast kinetic studies of plasmid DNA transfer in intact yeast cells mediated by electropulsation. BiochemBiophys Res Commun. 1995 Sep 25;214(3):825-32.

[6] Bonnafous P, Vernhes M, Teissie J, Gabriel B. The generation of reactive-oxygen species associated with long-lasting pulse-induced electropermeabilisation of mammalian cells is based on a non-destructive alteration of the plasma membrane. BiochimBiophysActa. 1999 Nov 9;1461(1):123-34.

[7] Heller R, Jaroszeski M, Atkin A, Moradpour D, Gilbert R, Wands J, et al. In vivo gene electroinjection and expression in rat liver. FEBS Lett. 1996 Jul 8;389(3):225-8.

[8] Mir LM, Bureau MF, Rangara R, Schwartz B, Scherman D. Long-term, high level in vivo gene expression after electric pulse-mediated gene transfer into skeletal muscle. C R AcadSci III. 1998 Nov;321(11):893-9.

[9] Aihara H, Miyazaki J. Gene transfer into muscle by electroporation in vivo. Nat Biotechnol. 1998 Sep;16(9):867-70.

[10] Rols MP, Delteil C, Golzio M, Dumond P, Cros S, Teissie J. In vivo electrically mediated protein and gene transfer in murine melanoma. Nat Biotechnol. 1998 Feb;16(2): 168-71.

[11] Suzuki T, Shin BC, Fujikura K, Matsuzaki T, Takata K. Direct gene transfer into rat liver cells by in vivo electroporation. FEBS Lett. 1998 Apr 3;425(3):436-40.

[12] Matsumoto T, Komori K, Shoji T, Kuma S, Kume M, Yamaoka T, et al. Successful and optimized in vivo gene transfer to rabbit carotid artery mediated by electronic pulse. Gene Ther. 2001 Aug;8(15):1174-9.

[13] Maruyama H, Ataka K, Higuchi N, Sakamoto F, Gejyo F, Miyazaki J. Skin-targeted gene transfer using in vivo electroporation. Gene Ther. 2001 Dec;8(23):1808-12.

[14] Jayankura M, Boggione C, Frisen C, Boyer O, Fouret P, Saillant G, et al. In situ gene transfer into animal tendons by injection of naked DNA and electrotransfer. J Gene Med. 2003 Jul;5(7):618-24.

[15] Harimoto K, Sugimura K, Lee CR, Kuratsukuri K, Kishimoto T. In vivo gene transfer methods in the bladder without viral vectors. Br J Urol. 1998 Jun;81(6):870-4.

[16] Blair-Parks K, Weston BC, Dean DA. High-level gene transfer to the cornea using electroporation. J Gene Med. 2002 Jan-Feb;4(1):92-100.

[17] 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.

[18] Lin CR, Tai MH, Cheng JT, Chou AK, Wang JJ, Tan PH, et al. Electroporation for direct spinal gene transfer in rats. NeurosciLett. 2002 Jan 4;317(1):1-4.

[19] Inoue T, Krumlauf R. An impulse to the brain--using in vivo electroporation. Nat Neurosci. 2001 Nov;4 Suppl:1156-8.

[20] Mir LM, Belehradek M, Domenge C, Orlowski S, Poddevin B, Belehradek J, Jr., et al. [Electrochemotherapy, a new antitumor treatment: first clinical trial]. C R AcadSci III. 1991;313(13):613-8.

[21] Gothelf A, Mir LM, Gehl J. Electrochemotherapy: results of cancer treatment using enhanced delivery of bleomycin by electroporation. Cancer Treat Rev. 2003 Oct;29(5): 371-87.

[22] Mir LM, Glass LF, Sersa G, Teissie J, Domenge C, Miklavcic D, et al. Effective treatment of cutaneous and subcutaneous malignant tumours by electrochemotherapy. Br J Cancer. 1998 Jun;77(12):2336-42.

[23] Rols MP, Bachaud JM, Giraud P, Chevreau C, Roche H, Teissie J. Electrochemotherapy of cutaneous metastases in malignant melanoma. Melanoma Res. 2000 Oct;10(5): 468-74.

[24] Rebersek M, Cufer T, Cemazar M, Kranjc S, Sersa G. Electrochemotherapy with cisplatin of cutaneous tumor lesions in breast cancer. Anticancer Drugs. 2004 Jul;15(6): 593-7.

[25] Sersa G, Miklavcic D, Cemazar M, Rudolf Z, Pucihar G, Snoj M. Electrochemotherapy in treatment of tumours. Eur J SurgOncol. 2008 Feb;34(2):232-40.

[26] Escoffre JM, Portet T, Wasungu L, Teissie J, Dean D, Rols MP. What is (still not) known of the mechanism by which electroporation mediates gene transfer and expression in cells and tissues.MolBiotechnol. 2009 Mar;41(3):286-95.

[27] Faurie C, Phez E, Golzio M, Vossen C, Lesbordes JC, Delteil C, et al. Effect of electric field vectoriality on electrically mediated gene delivery in mammalian cells. BiochimBiophysActa. 2004 Oct 11;1665(1-2):92-100.

[28] Rols MP, Delteil C, Golzio M, Teissie J. Control by ATP and ADP of voltage-induced mammalian-cell-membrane permeabilization, gene transfer and resulting expression. Eur J Biochem. 1998 Jun 1;254(2):382-8.

[29] Teissie J, Rols MP. An experimental evaluation of the critical potential difference inducing cell membrane electropermeabilization. Biophys J. 1993 Jul;65(1):409-13.

[30] Gabriel B, Teissie J. Direct observation in the millisecond time range of fluorescent molecule asymmetrical interaction with the electropermeabilized cell membrane. Biophys J. 1997 Nov;73(5):2630-7.

[31] Klenchin VA, Sukharev SI, Serov SM, Chernomordik LV, Chizmadzhev Yu A. Electrically induced DNA uptake by cells is a fast process involving DNA electrophoresis. Biophys J. 1991 Oct;60(4):804-11.

[32] 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 Jul;58(1):13-9.

[33] deGennes PG. Passive entry of a DNA molecule into a small pore. ProcNatlAcadSci U S A. 1999 Jun 22;96(13):7262-4.

[34] Sukharev SI, Klenchin VA, Serov SM, Chernomordik LV, Chizmadzhev Yu A. Electroporation and electrophoretic DNA transfer into cells. The effect of DNA interaction with electropores. Biophys J. 1992 Nov;63(5):1320-7.

[35] Golzio M, Teissie J, Rols MP. Direct visualization at the single-cell level of electrically mediated gene delivery. ProcNatlAcadSci U S A. 2002 Feb 5;99(3):1292-7.

[36] Golzio M, Rols MP, Teissie J. In vitro and in vivo electric field-mediated permeabilization, gene transfer, and expression. Methods. 2004 Jun;33(2):126-35.

[37] Belehradek J, Jr., Orlowski S, Ramirez LH, Pron G, Poddevin B, Mir LM. Electropermeabilization of cells in tissues assessed by the qualitative and quantitative electroloading of bleomycin. BiochimBiophysActa. 1994 Feb 23;1190(1):155-63.

[38] Gehl J, Sorensen TH, Nielsen K, Raskmark P, Nielsen SL, Skovsgaard T, et al. In vivo electroporation of skeletal muscle: threshold, efficacy and relation to electric field distribution. BiochimBiophysActa. 1999 Aug 5;1428(2-3):233-40.

[39] Mir LM, Bureau MF, Gehl J, Rangara R, Rouy D, Caillaud JM, et al. High-efficiency gene transfer into skeletal muscle mediated by electric pulses. ProcNatlAcadSci U S A. 1999 Apr 13;96(8):4262-7.

[40] Bureau MF, Gehl J, Deleuze V, Mir LM, Scherman D. Importance of association between permeabilization and electrophoretic forces for intramuscular DNA electrotransfer. BiochimBiophysActa. 2000 May 1;1474(3):353-9.

[41] Satkauskas S, Bureau MF, Puc M, Mahfoudi A, Scherman D, Miklavcic D, et al. Mechanisms of in vivo DNA electrotransfer: respective contributions of cell electropermeabilization and DNA electrophoresis. MolTher. 2002 Feb;5(2):133-40.

[42] Paturneau-Jouas M, Parzy E, Vidal G, Carlier PG, Wary C, Vilquin JT, et al. Electrotransfer at MR imaging: tool for optimization of gene transfer protocols--feasibility study in mice. Radiology. 2003 Sep;228(3):768-75.

[43] Satkauskas S, Bureau MF, Mahfoudi A, Mir LM. Slow accumulation of plasmid in muscle cells: supporting evidence for a mechanism of DNA uptake by receptor-mediated endocytosis. MolTher. 2001 Oct;4(4):317-23.

[44] Cappelletti M, Zampaglione I, Rizzuto G, Ciliberto G, La Monica N, Fattori E. Gene electro-transfer improves transduction by modifying the fate of intramuscular DNA. J Gene Med. 2003 Apr;5(4):324-32.

[45] Bureau MF, Naimi S, Torero Ibad R, Seguin J, Georger C, Arnould E, et al. Intramuscular plasmid DNA electrotransfer: biodistribution and degradation. BiochimBiophysActa. 2004 Jan 20;1676(2):138-48.

[46] Burgain A, Scherman D, Bigey P. Electrotransfert : concept et historique. L'Actualité-Chimique. 2009;327*328:68-74.

[47] 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 Apr 16;22(13-14):1820-5.

[48] Tollefsen S, Vordermeier M, Olsen I, Storset AK, Reitan LJ, Clifford D, et al. DNA injection in combination with electroporation: a novel method for vaccination of farmed ruminants. Scand J Immunol. 2003 Mar;57(3):229-38.

[49] Rambabu KM, Rao SH, Rao NM. Efficient expression of transgenes in adult zebrafish by electroporation. BMC Biotechnol. 2005;5:29.

[50] Miklavcic D, Semrov D, Mekid H, Mir LM. A validated model of in vivo electric field distribution in tissues for electrochemotherapy and for DNA electrotransfer for gene therapy. BiochimBiophysActa. 2000 Sep 1;1523(1):73-83.

[51] Bloquel C, Fabre E, Bureau MF, Scherman D. Plasmid DNA electrotransfer for intracellular and secreted proteins expression: new methodological developments and applications. J Gene Med. 2004 Feb;6Suppl 1:S11-23.

[52] Mathiesen I. Electropermeabilization of skeletal muscle enhances gene transfer in vivo. Gene Ther. 1999 Apr;6(4):508-14.

[53] Taylor J, Babbs CF, Alzghoul MB, Olsen A, Latour M, Pond AL, et al. Optimization of ectopic gene expression in skeletal muscle through DNA transfer by electroporation. BMC Biotechnol. 2004 May 18;4:11.

[54] Maccarrone M, Bladergroen MR, Rosato N, Finazzi Agro AF. Role of lipid peroxidation in electroporation-induced cell permeability. BiochemBiophys Res Commun. 1995 Apr 17;209(2):417-25.

[55] Hartikka J, Sukhu L, Buchner C, Hazard D, Bozoukova V, Margalith M, et al. Electroporation-facilitated delivery of plasmid DNA in skeletal muscle: plasmid dependence of muscle damage and effect of poloxamer 188. MolTher. 2001 Nov;4(5):407-15.

[56] Rubenstrunk A, Mahfoudi A, Scherman D. Delivery of electric pulses for DNA electrotransfer to mouse muscle does not induce the expression of stress related genes. Cell BiolToxicol. 2004 Feb;20(1):25-31.

[57] Trollet C, Scherman D, Bigey P. Delivery of DNA into muscle for treating systemic diseases: advantages and challenges. Methods Mol Biol. 2008;423:199-214.

[58] Schertzer JD, Lynch GS. Plasmid-based gene transfer in mouse skeletal muscle by electroporation. Methods Mol Biol. 2008;433:115-25.

[59] Mennuni C, Calvaruso F, Zampaglione I, Rizzuto G, Rinaudo D, Dammassa E, et al. Hyaluronidase increases electrogene transfer efficiency in skeletal muscle. Hum Gene Ther. 2002 Feb 10;13(3):355-65.

[60] Favre D, Cherel Y, Provost N, Blouin V, Ferry N, Moullier P, et al. Hyaluronidase enhances recombinant adeno-associated virus (rAAV)-mediated gene transfer in the rat skeletal muscle. Gene Ther. 2000 Aug;7(16):1417-20.

[61] McMahon JM, Signori E, Wells KE, Fazio VM, Wells DJ. Optimisation of electrotransfer of plasmid into skeletal muscle by pretreatment with hyaluronidase -- increased expression with reduced muscle damage. Gene Ther. 2001 Aug;8(16):1264-70.

[62] Davis HL, Whalen RG, Demeneix BA. Direct gene transfer into skeletal muscle in vivo: factors affecting efficiency of transfer and stability of expression. Hum Gene Ther. 1993 Apr;4(2):151-9.

[63] Nicol F, Wong M, MacLaughlin FC, Perrard J, Wilson E, Nordstrom JL, et al. Poly-L-glutamate, an anionic polymer, enhances transgene expression for plasmids delivered by intramuscular injection with in vivo electroporation. Gene Ther. 2002 Oct; 9(20):1351-8.

[64] Mir LM, Moller PH, Andre F, Gehl J. Electric pulse-mediated gene delivery to various animal tissues. Adv Genet. 2005;54:83-114.

[65] Goldspink G. Skeletal muscle as an artificial endocrine tissue. Best Pract Res ClinEndocrinolMetab. 2003 Jun;17(2):211-22.

[66] Escoffre JM, Debin A, Reynes JP, Drocourt D, Tiraby G, Hellaudais L, et al. Long-lasting in vivo gene silencing by electrotransfer of shRNA expressing plasmid. Technol Cancer Res Treat. 2008 Apr;7(2):109-16.

[67] Kanitakis J. Anatomy, histology and immunohistochemistry of normal human skin. Eur J Dermatol. 2002 Jul-Aug;12(4):390-9; quiz 400-1.

[68] Denet AR, Vanbever R, Preat V. Skin electroporation for transdermal and topical delivery. Adv Drug Deliv Rev. 2004 Mar 27;56(5):659-74.

[69] Jadoul A, Bouwstra J, Preat VV. Effects of iontophoresis and electroporation on the stratum corneum. Review of the biophysical studies. Adv Drug Deliv Rev. 1999 Jan 4;35(1):89-105.

[70] Pavselj N, Preat V. DNA electrotransfer into the skin using a combination of one high- and one low-voltage pulse. J Control Release. 2005 Sep 2;106(3):407-15.

[71] Zhang L, Nolan E, Kreitschitz S, Rabussay DP. Enhanced delivery of naked DNA to the skin by non-invasive in vivo electroporation. BiochimBiophysActa. 2002 Aug 15;1572(1):1-9.

[72] Dujardin N, Staes E, Kalia Y, Clarys P, Guy R, Preat V. In vivo assessment of skin electroporation using square wave pulses. J Control Release. 2002 Feb 19;79(1-3): 219-27.

[73] Cemazar M, Golzio M, Sersa G, Escoffre JM, Coer A, Vidic S, et al. Hyaluronidase and collagenase increase the transfection efficiency of gene electrotransfer in various murine tumors. Hum Gene Ther. 2012 Jan;23(1):128-37.

[74] Trollet C, Pereira Y, Burgain A, Litzler E, Mezrahi M, Seguin J, et al. Generation of high-titer neutralizing antibodies against botulinum toxins A, B, and E by DNA electrotransfer. Infect Immun. 2009 May;77(5):2221-9.

[75] Adachi O, Nakano A, Sato O, Kawamoto S, Tahara H, Toyoda N, et al. Gene transfer of Fc-fusion cytokine by in vivo electroporation: application to gene therapy for viral myocarditis. Gene Ther. 2002 May;9(9):577-83.

[76] Daud AI, DeConti RC, Andrews S, Urbas P, Riker AI, Sondak VK, et al. Phase I trial of interleukin-12 plasmid electroporation in patients with metastatic melanoma. J ClinOncol. 2008 Dec 20;26(36):5896-903.

[77] Heller LC, Heller R. Electroporation gene therapy preclinical and clinical trials for melanoma. Curr Gene Ther. 2010 Aug;10(4):312-7.

[78] Bettan M, Ivanov MA, Mir LM, Boissiere F, Delaere P, Scherman D. Efficient DNA electrotransfer into tumors. Bioelectrochemistry. 2000 Sep;52(1):83-90.

[79] Bloquel C, Bessis N, Boissier MC, Scherman D, Bigey P. Gene therapy of collagen-induced arthritis by electrotransfer of human tumor necrosis factor-alpha soluble receptor I variants. Hum Gene Ther. 2004 Feb;15(2):189-201.

[80] Gollins H, McMahon J, Wells KE, Wells DJ. High-efficiency plasmid gene transfer into dystrophic muscle. Gene Ther. 2003 Mar;10(6):504-12.

[81] Tanaka T, Ichimaru N, Takahara S, Yazawa K, Hatori M, Suzuki K, et al. In vivo gene transfer of hepatocyte growth factor to skeletal muscle prevents changes in rat kidneys after 5/6 nephrectomy. Am J Transplant. 2002 Oct;2(9):828-36.

[82] Bakker JM, Bleeker WK, Parren PW. Therapeutic antibody gene transfer: an active approach to passive immunity. MolTher. 2004 Sep;10(3):411-6.

[83] Perez N, Bigey P, Scherman D, Danos O, Piechaczyk M, Pelegrin M. Regulatable systemic production of monoclonal antibodies by in vivo muscle electroporation. Genet Vaccines Ther. 2004 Mar 23;2(1):2.

[84] Bernal SD, Ona ET, Riego-Javier A, R DEV, Cristal-Luna GR, Laguatan JB, et al. Anticancer immune reactivity and long-term survival after treatment of metastatic ovarian cancer with dendritic cells. OncolLett. Jan;3(1):66-74.

[85] Goto T, Nishi T, Kobayashi O, Tamura T, Dev SB, Takeshima H, et al. Combination electro-gene therapy using herpes virus thymidine kinase and interleukin-12 expression plasmids is highly efficient against murine carcinomas in vivo. MolTher. 2004 Nov;10(5):929-37.

[86] Shao D, Zeng Q, Fan Z, Li J, Zhang M, Zhang Y, et al. Monitoring HSV-TK/ganciclovir cancer suicide gene therapy using CdTe/CdS core/shell quantum dots. Biomaterials. 2012 Jun;33(17):4336-44.

[87] 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 Jan;9(1):16-27.

[88] Prabha S, Sharma B, Labhasetwar V. Inhibition of tumor angiogenesis and growth by nanoparticle-mediated p53 gene therapy in mice. Cancer Gene Ther. 2012 Aug;19(8): 530-7.

[89] Finetti F, Terzuoli E, Bocci E, Coletta I, Polenzani L, Mangano G, et al. Pharmacological inhibition of microsomal prostaglandin e synthase-1 suppresses epidermal growth factor receptor-mediated tumor growth and angiogenesis. PLoS One. 2012;7(7):e40576.

[90] Hanari N, Matsubara H, Hoshino I, Akutsu Y, Nishimori T, Murakami K, et al. Combinatory gene therapy with electrotransfer of midkine promoter-HSV-TK and interleukin-21. Anticancer Res. 2007 Jul-Aug;27(4B):2305-10.

[91] Cemazar M, Jarm T, Sersa G. Cancer electrogene therapy with interleukin-12. Curr Gene Ther. 2010 Aug;10(4):300-11.

[92] Tamura T, Nishi T, Goto T, Takeshima H, Ushio Y, Sakata T. Combination of IL-12 and IL-18 of electro-gene therapy synergistically inhibits tumor growth. Anticancer Res. 2003 Mar-Apr;23(2B):1173-9.

[93] Craig R, Cutrera J, Zhu S, Xia X, Lee YH, Li S. Administering plasmid DNA encoding tumor vessel-anchored IFN-alpha for localizing gene product within or into tumors. MolTher. 2008 May;16(5):901-6.

[94] Zhang L, Zhao L, Zhao D, Lin G, Guo B, Li Y, et al. Inhibition of tumor growth and induction of apoptosis in prostate cancer cell lines by overexpression of tissue inhibitor of matrix metalloproteinase-3. Cancer Gene Ther. 2010 Mar;17(3):171-9.

[95] Zhu LP, Yin Y, Xing J, Li C, Kou L, Hu B, et al. Therapeutic efficacy of Bifidobacteriumlongum-mediated human granulocyte colony-stimulating factor and/or endostatin combined with cyclophosphamide in mouse-transplanted tumors. Cancer Sci. 2009 Oct;100(10):1986-90.

[96] Chudley L, McCann K, Mander A, Tjelle T, Campos-Perez J, Godeseth R, et al. DNA fusion-gene vaccination in patients with prostate cancer induces high-frequency CD8(+) T-cell responses and increases PSA doubling time. Cancer ImmunolImmunother. 2012 May 22;2012:22.

[97] Low L, Mander A, McCann K, Dearnaley D, Tjelle T, Mathiesen I, et al. DNA vaccination with electroporation induces increased antibody responses in patients with prostate cancer. Hum Gene Ther. 2009 Nov;20(11):1269-78.

[98] Pichavant C, Chapdelaine P, Cerri DG, Bizario JC, Tremblay JP. Electrotransfer of the full-length dog dystrophin into mouse and dystrophic dog muscles. Hum Gene Ther. Nov;21(11):1591-601.

[99] Kreiss P, Bettan M, Crouzet J, Scherman D. Erythropoietin secretion and physiological effect in mouse after intramuscular plasmid DNA electrotransfer. J Gene Med. 1999 Jul-Aug;1(4):245-50.

[100] Rizzuto G, Cappelletti M, Maione D, Savino R, Lazzaro D, Costa P, et al. Efficient and regulated erythropoietin production by naked DNA injection and muscle electroporation. ProcNatlAcadSci U S A. 1999 May 25;96(11):6417-22.

[101] Payen E, Bettan M, Henri A, Tomkiewitcz E, Houque A, Kuzniak I, et al. Oxygen tension and a pharmacological switch in the regulation of transgene expression for gene therapy. J Gene Med. 2001 Sep-Oct;3(5):498-504.

[102] Payen E, Bettan M, Rouyer-Fessard P, Beuzard Y, Scherman D. Improvement of mouse beta-thalassemia by electrotransfer of erythropoietin cDNA. ExpHematol. 2001 Mar;29(3):295-300.

[103] Dalle B, Henri A, Rouyer-Fessard P, Bettan M, Scherman D, Beuzard Y, et al. Dimeric erythropoietin fusion protein with enhanced erythropoietic activity in vitro and in vivo. Blood. 2001 Jun 15;97(12):3776-82.

[104] Livnah O, Stura EA, Middleton SA, Johnson DL, Jolliffe LK, Wilson IA. Crystallographic evidence for preformed dimers of erythropoietin receptor before ligand activation. Science. 1999 Feb 12;283(5404):987-90.

[105] Fabre EE, Bigey P, Beuzard Y, Scherman D, Payen E. Careful adjustment of Epo nonviral gene therapy for beta-thalassemicanaemia treatment. Genet Vaccines Ther. 2008;6:10.

[106] Gothelf A, Hojman P, Gehl J. Therapeutic levels of erythropoietin (EPO) achieved after gene electrotransfer to skin in mice. Gene Ther. 2010 Sep;17(9):1077-84.

[107] Marshall WG, Jr., Boone BA, Burgos JD, Gografe SI, Baldwin MK, Danielson ML, et al. Electroporation-mediated delivery of a naked DNA plasmid expressing VEGF to the porcine heart enhances protein expression. Gene Ther. 2010 Mar;17(3):419-23.

[108] Ayuni EL, Gazdhar A, Giraud MN, Kadner A, Gugger M, Cecchini M, et al. In vivo electroporation mediated gene delivery to the beating heart. PLoS One. 2010;5(12):e14467.

[109] Dean D. DNA electrotransfer to the skin: a highly translatable approach to treat peripheral artery disease. Gene Ther. 2010 Jun;17(6):691.

[110] Bloquel C, Bejjani R, Bigey P, Bedioui F, Doat M, BenEzra D, et al. Plasmid electrotransfer of eye ciliary muscle: principles and therapeutic efficacy using hTNF-alpha soluble receptor in uveitis. Faseb J. 2006 Feb;20(2):389-91.

[111] Touchard E, Bloquel C, Bigey P, Kowalczuk L, Jonet L, Thillaye-Goldenberg B, et al. Effects of ciliary muscle plasmid electrotransfer of TNF-alpha soluble receptor variants in experimental uveitis. Gene Ther. 2009 Jul;16(7):862-73.

[112] Touchard E, Berdugo M, Bigey P, El Sanharawi M, Savoldelli M, Naud MC, et al. SuprachoroidalElectrotransfer: A Nonviral Gene Delivery Method to Transfect the Choroid and the Retina Without Detaching the Retina. MolTher. 2012 Jan 17.

[113] Hojman P, Brolin C, Gissel H, Brandt C, Zerahn B, Pedersen BK, et al. Erythropoietin over-expression protects against diet-induced obesity in mice through increased fat oxidation in muscles. PLoS One. 2009;4(6):e5894.

[114] Bruce CR, Hoy AJ, Turner N, Watt MJ, Allen TL, Carpenter K, et al. Overexpression of carnitine palmitoyltransferase-1 in skeletal muscle is sufficient to enhance fatty acid oxidation and improve high-fat diet-induced insulin resistance. Diabetes. 2009 Mar;58(3):550-8.

[115] Kanzleiter T, Preston E, Wilks D, Ho B, Benrick A, Reznick J, et al. Overexpression of the orphan receptor Nur77 alters glucose metabolism in rat muscle cells and rat muscle in vivo. Diabetologia. 2010 Jun;53(6):1174-83.

[116] Quinn A, Jiang W, Velaz-Faircloth M, Cobb AJ, Henry SC, Frothingham R. In vivo protein expression and immune responses generated by DNA vaccines expressing mycobacterial antigens fused with a reporter protein. Vaccine. 2002 Aug 19;20(25-26): 3187-92.

[117] Kirman JR, Seder RA. DNA vaccination: the answer to stable, protective T-cell memory? CurrOpinImmunol. 2003 Aug;15(4):471-6.

[118] Widera G, Austin M, Rabussay D, Goldbeck C, Barnett SW, Chen M, et al. Increased DNA vaccine delivery and immunogenicity by electroporation in vivo. J Immunol. 2000 May 1;164(9):4635-40.

[119] Zucchelli S, Capone S, Fattori E, Folgori A, Di Marco A, Casimiro D, et al. Enhancing B- and T-cell immune response to a hepatitis C virus E2 DNA vaccine by intramuscular electrical gene transfer. J Virol. 2000 Dec;74(24):11598-607.

[120] Tollefsen S, Tjelle T, Schneider J, Harboe M, Wiker H, Hewinson G, et al. Improved cellular and humoral immune responses against Mycobacterium tuberculosis antigens after intramuscular DNA immunisation combined with muscle electroporation. Vaccine. 2002 Sep 10;20(27-28):3370-8.

[121] Bachy M, Boudet F, Bureau M, Girerd-Chambaz Y, Wils P, Scherman D, et al. Electric pulses increase the immunogenicity of an influenza DNA vaccine injected intramuscularly in the mouse. Vaccine. 2001 Feb 8;19(13-14):1688-93.

[122] Tjelle TE, Corthay A, Lunde E, Sandlie I, Michaelsen TE, Mathiesen I, et al. Monoclonal antibodies produced by muscle after plasmid injection and electroporation. MolTher. 2004 Mar;9(3):328-36.

IntechOpen