Chapter 2

Electrically Mediated Gene Delivery : Basic and Translational Concepts

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1. Introduction

Naked plasmid (pDNA) electrotransfer is an approach for gene transfer that is very efficient for in vitro studies since its introduction in 1982. It was extended for a use in vivo in a perspective for clinical gene therapy.

This chapter is a review on the state of the art. The molecular processes bringing the pDNA transfer and expression will be described. A critical view of the barriers preventing an efficient level of expression is given. This gives the tools to design the relevant protocols for the use on animals and the potential clinical trials.

Delivery of naked plasmids (pDNA) in tissue to obtain gene expression was facing the limit of a poor level of expression [1]. But the clinical advantage was that it was a safe approach for the patient. Improvement of the delivery and the resulting expression was known to be obtained at the cellular level by applying electric field pulses to the cell-pDNA mixture [2]. It was shown that this boost in expression could be obtained on tissues [3]. During the last 15 years, many developments in this approach have been performed and Electrotransfection is now considered as a perspective for gene therapies [4-9]. A Phase I clinical trial using gene therapy by electrically mediated delivery has been performed (www.clinicaltrials.gov identifier NCT00323206)[10]. More recent data showed that the delivery and expression can be obtained on very sensitive organs [11-13]. Electropulsation mediated gene delivery appears now to be one of the effective contributors for the success of gene therapy

2. Basic processes

2.1. Electropermeabilization

Biological membrane cohesion is known for almost 40 years to be affected by external electric field pulses [14]. A transient and reversible membrane permeabilization (Electropermeabilization) results from a controlled application of electric pulses to cells [15]. This can be induced not only in vitro but on living tissues as well. The key feature is that the structural transition is obtained only when the external field is larger than a critical value. A physical targeting of the effect is therefore present in tissues. This improves the safety of the approach as only a limited volume of the tissue is affected. This metastable membrane structural organization remains poorly understood. New properties are brought to the cell plasma membrane that, besides being permeabilized, becomes fusogenic and allows exogenous proteins to be inserted in it. Electropermeabilization is used to introduce a large variety of molecules into many different cells *in vitro* [16, 17]. The molecular transports, that result, are either due to an electrophoretic drift or/and to a concentration driven diffusion [18, 19]. The practical use for drug delivery remains rather empirical but results from more than 20 years of trials. Clinical applications of the method are now routinely used in many oncology centers (more than 100 in Europe only) as results from the EU funded Cliniporator and ESOPE programs. A local antitumoral drug delivery to patients (electrochemotherapy) results from the direct application of electric pulses to the patient [20-27]. In Europe, the treatment has been approved and patients can be reimbursed,

2.2. Electrotransfection

The most frequent application of electric field induced membrane permeabilization is the transfer and expression of gene into mammalian cells. Plasmid DNA (pDNA) can be transferred and expressed in mammalian cells when electropermeabilization is triggered [2]. This a complex process that involves not only the transport of pDNA into the cytoplasm, but also depends on subsequent cellular processes [27]. The transfer of naked DNA plasmid and the expression of the gene of interest are enhanced by electropulsation into different tissues, including the skeletal muscle [28, 29], liver [3,30], skin [31, 32], lungs [33] and tumors [34, 35]. The transfection efficiency of this physical method *in vivo* must still be improved compared to the viral vectors. But it is obtained with naked pDNA avoiding the biological risks associated to the viral methods. Furthermore there is no theoretical restriction on the size of the pDNA to transfer. As a result, due to its easiness to perform, to the very fast expression after electric pulse delivery, reproducibility, limited costs (of the technology and logistics) and safety, gene electrotransfer is an attractive technology of gene therapy for clinical application. This is well illustrated by the increasing number of reviews covering the pre-clinical developments of the approach [4-9, 36].

As mentioned above, one of main limits of the widespread use of electropermeabilization is that very few is known on the biophysical mechanisms supporting the reorganization of the cell membrane (pore, electropore, defects?). The molecular target of the field effect remains

unclear [15]. The other main limit in gene electrotransfer is that the transport is not only across the plasma membrane but must target the nucleus volume.

The present review focuses on the processes supporting gene electrotransfer in vitro and their implications for the clinical applications. The events occurring before, during, after pulse application leading to gene electrotransfer will be described. Theoretical considerations about membrane structures involved in the plasmid uptake will be described in a (very) critical manner as very few direct experimental data are available.

3. Theories of DNA plasmid electroentry

Although the first pioneering report on gene electrotransfer in cells was published 30 years ago by E. Neumann, the molecular basis behind the process of gene electrotransfer is still highly debated.

3.1. The sliding model

Most theories are based on the DNA sliding model [37]. Several steps are predicted: entry, electrophoretic translocation as long as the field was present, diffusion after the pulse delivery. An interesting simulation with predictive conclusions has just been published [38]. The DNA translocation was through a putative "electropore" assuming that the DNA was double stranded but linear (i.e open) meaning that the length was 2.4 μm. Indeed the sliding model needs the DNA to be linear to allow the "binding" of one end to the "electropore" entry. Experimental results just showed that the closed form was more effective for expression [39]. One speculative hypothesis in the simulation was that it always assumed that the binding step (entry) of the DNA to the "electropore" was present before the simulation started. De Gennes predicted that it was a very limiting step in the process (a crucial moment) when a chain end faces the electropore and enters it against the strong friction coefficient against the "pore" sides. A black box remains the "electropore".

3.2. The electropore

The major hypothesis in the sliding model is that "pores" must be present. Krassowska's model supports the simplest mechanism, in which plasmid enters the 5 nm thick membrane through stable electropores (up to 20 nm in diameter)[40, 41]. The electrically induced defects result from the field associated membrane potential changes. It predicts a post-pulse growth of "macropores" on the sub-second time scale fairly consistent with experimental evidence on pure lipid vesicles [42]. This model predicts "electropores" large enough to permit the plasmid uptake (under a linear form). These "electropores" are supposed to remain open for the entire duration of electrotransfer providing adequate time for the plasmid to enter the cell [43]. Indeed in Lin's simulation [38], they may remain present on a much longer duration than the pulse as a diffusion might follow a partial electrotransfer.

This model remains very attractive in spite of the existence of many experimental contradictions. Indeed, until now, no study made it possible to visualize these membrane pores. This

validation appears impossible [27]. Moreover, the resealing time of pores appears to be shorter in this lipidic model than in experiments on cells (e.g. seconds rather than minutes) [44-47]. The conclusion is that pDNA transport across the membrane is always very fast. To date, theoretical models could predict stable pores of only a few nanometers in radius; larger pores are unstable while they are needed for the sliding pDNA transfer [48, 49]. These models are confirmed by some experiments, in which high-voltage pulses a few microseconds long are used that are supposed to have created a large number of very small pores (radii of about 1 nm, i.e. the size of a few phospholipids cluster) [50]. To reconcile these results with the experimental evidence of plasmid translocation after electropulsation, some researchers postulated that plasmid entry into cells relies on the plasmid/membrane interactions, which may be facilitated by a coalescence of many small, 1 nm defects [51-54, 43]. The slow transport of DNA across the electropermeabilized membrane reflects a highly interactive electrotransfer, where many small lipid defects coalesced into large DNA-lipids assemblies where the transmembrane transport occurs [55].

3.3. Electrophoresis across the micellar structures

Other data report that gene electrotransfer through lipid bilayer could be mediated by transient complexes between plasmid and specific lipids in the edges of elongated, electropercolated hydrophilic membrane associated micellar structures [56]. The plasmid association with a lipid bilayer results in a facilitated transport of small ions. A locally conductive plasmid/lipid interaction zone is induced where parts of the plasmid may be inserted in the bilayer. Plasmid is transiently inserted in, and then electrophoretically pulled through the permeabilized zones onto the other membrane side [57]. With such a model, in the case of mammalian cells, the resting potential difference should be the driving force for plasmid translocation after the pulse induced insertion. The external field is only used to electrophoretically accumulate the pDNA on the cell surface. This has not been checked yet.

3.4. An electrophoretic transfer

Previous works suggested that electric pulses induce the membrane permeabilization, then plasmid molecules are concentrated near the membrane surface and pushed through by electrophoretic forces [58-60, 54]. The plasmid may interact with electropermeabilized membrane by three possible ways: (a) the plasmid coil is aligned in an electric field, and at the appropriate pulse polarity it moves toward the permeabilized membrane. Transfer is dependent on electrophoretic forces and is complete at the end of the pulse. Post pulse cell treatment should not affect the efficiency of transfer.

If the electrophoretic forces are the only driving forces of the plasmid transfer into the cell, similar transfection efficiencies should be obtained for equal E.T values (i.e. E, field strength and T, pulse duration). This is not supported by the experiments [61] When the E.N.T value is constant, transfection rate depends preferentially on T [52]. Therefore, the electrophoretic migration cannot be the only driving force of the plasmid transfer into the cells but clearly supports the formation of aggregates. Trypsin treatment of cells at 10 min post electrotransfection stripped off membrane-bound pDNA and resulted in a significant reduction in

transfection, indicating that the time period for complete cellular uptake of pDNA (between 10 and 40 min) far exceeded the lifetime of electric field-induced transient pores (10 msec) in the cell membrane [62]. In addition, in the case of CHO cells, plasmid remains accessible to DNAase I in the minute, which follows the end of electropulsation. This shows that the plasmid transfer inside the cell occurs after the electropulsation [17].

3.5. In silico electrotransfer

A molecular dynamic approach gives a mechanism, in which plasmids do not translocate across the membrane during the electropulsation [63]. The DNA/lipid system simulation was undertaken starting from a well-equilibrated 12bp-DNA duplex placed near a model POPC bilayer. The perturbation of the system under a 1.0 $V.nm^{-1}$ transverse electric field (i.e. a transmembrane voltage of 5 V !) is followed during 2 ns. Under this high electric field, the DNA duplex diffuses towards the interior of the bilayer only after the creation of a pore beneath it, and within the same timescale, it remains at the interfacial region when no pore is present. Diffusion of the strand toward the interior of the membrane leads to a DNA/lipid complex in which the lipid head groups encapsulate the strand. The dipole carried by the zwitterionic phosphatidylcholine groups of the lipids is known to be efficient for neutralizing the charges carried by the DNA [64]. Such interactions between the plasmid and the lipids contribute to the effective screening of DNA charges and therefore to the stabilization of the complex. One should not forget that electropulsation-mediated gene delivery concerns much larger supercoiled plasmids than the 12 bp construct considered in the MD simulation.

3.6. Endocytosis

Most methods for chemically mediated gene transfer described the transport as an endocytotic complex formation between the DNA vesicles and the cell surface. Several studies investigated if this can occur in electrotransfection. Treatment of cells with three endocytic inhibitors (chlorpromazine, genistein, dynasore) yielded substantial and statistically significant reductions in the electrotransfection mediated expression [62]. These findings suggest that electrotransfection depends on endocytosis of membrane-bound pDNA. [65]. Colocalization studies with endocytotic markers under a microscope showed that pDNA is internalized with concomitant clathrin- and caveolin/raft-mediated endocytosis [66]. But this cannot explain how the pDNA is released from the endocytic vesicles and why free PDNA was observed in the cytoplasm a few minutes after the pulse delivery [67]. A direct assay of the formation of endocytic vesicles brought the conclusion that endocytosis was not stimulated by applying electric pulses with intensities above the threshold value for gene electrotransfer. The conclusion was that electro-endocytosis is not a crucial mechanism for gene electrotransfer [68].

3.7. The multistep model

PDNA electrotransfer was observed at the single cell level by digitized high resolution fluorescence microscopy [67]. The introduction of DNA only occurs in the part of the membrane facing the cathode and requires a number of consecutive steps: electrophoretic migration of DNA towards the cell, DNA insertion into the membrane, translocation across the mem-

brane, migration of DNA towards the nucleus and, finally, transfer of DNA across the nuclear envelope. Only localized parts of the cell membrane brought to the permeabilized state are competent for transfer. The transport of plasmid follows an "anchoring step", connecting the plasmid to the permeabilized membrane, that takes place during the pulse. During the first pulsation, plasmids are electrophoretically drifted and interact with a limited number of sites on the membrane. These sites become highly conductive and attract the field lines giving an electrophoretic local accumulation [69]. PDNAs form a limited number of aggregates on the cell surface. Their sizes increase during the pulses or with successive pulses but not their number [70]. Transfer of membrane bound plasmids is certainly a complex process, that is not occurring during but after the pulse delivery. Two classes of DNA/membrane interactions result from the pulse: (i) a metastable DNA/membrane complex from which the DNA can leave and return to external medium and (ii) a stable DNA/membrane. Only DNA belonging to the second class may be effective for transmembrane transport and the resulting gene expression [71]. Nevertheless this model shows that the plasmid is stabilized in the millisecond following the pulse in the membrane core after electropulsation (in agreement with the overall experimentally observed process of DNA translocation).

4. The cytoplasmic transfer

Transfer from the membrane to the nucleus is mediated by the cell (cytoskeleton with molecular motors?). The final step should take place through the nuclear pore complex [72-74]. No direct biophysical method to alter the nuclear envelope or pore has been reported (yet). But imaging methods [75, 76] support the occurrence of a direct effect of the field on organelles [77].

For non-viral gene delivery to be successful, plasmids must move through the cytoplasm to the nucleus in order to be transcribed. 2 steps are therefore present involving 2 classes of barriers. The cytoskeletal meshwork prevents pDNA (larger than 1 kb) movement in the cytoplasm. Actin patches colocalizing with the DNA at the plasma membrane were observed several minutes after pulse delivery with characteristics similar to those of the DNA aggregates, that are formed during the early stages of electrotransfection [78]. The microtubule network is required for directed plasmid trafficking to the nucleus [79]. Microtubule–DNA interactions can be enhanced due to sequence specificity with promoters containing binding sites for cyclic AMP response-element binding protein (CREB), such as the cytomegalovirus immediate early promoter (CMViep). Insertion of cytoplasmic adapter proteins transcription factors (TFs) binding sites within plasmids permits cytoplasmic trafficking of plasmids and an effective expression.

NLS sequences can help for the transfer inside the nucleus. In non-dividing cells, the nuclear envelope is an especially problematic hurdle to gene transfer. A successful approach is in modifying plasmid (pDNA) vectors to enhance nuclear import through the Nuclear Pore Complex [80]. Proteomics tools have been used to study DNA nuclear entry telling that Transcription factor-binding sites promote DNA nuclear translocation and Cell-specific transcription factors drive cell-specific DNA nuclear entry [81]. NLS peptides or nuclear proteins complexed with plasmids may enhance DNA nuclear translocation.

5. PDNA under electrotransfection conditions

5.1. Complexities in the determination of pDNA size evaluation

The size of the plasmid can be a significant modulator of the efficiency of transfer. The sliding model assumed that the pDNA was linear. This is not relevant of the experiments where a closed form was used in almost all reported cases. The gyration volume appears more appropriated. It is known that this diameter is highly sensitive to the compaction factors that are present. A tightly packed form is found in viral capsids. During the last 20 years, biotechnologists have been playing with chemical additives to obtain more compact forms. Indeed adding NaCl and/or MgCl2 is affecting the diameters. In [82] the authors wrote: "conformational and thermodynamic properties of supercoiled DNA depend strongly on ionic conditions. The effective double-helix diameter increases from 3 to 15 nm as the salt concentration is reduced from 1.00 to 0.01 M.". It was later observed with pUC18 (2686 bp), in dilute aqueous solution at salt concentrations between 0 and 1.5 M Na+ in 10 mM Tris, that the superhelix diameter from the simulated conformations decreased from 18.0 +/- 1.5 nm at 10 mM to 9.4 +/- 1.5 nm at 100 mM salt concentration[83]. This value did not significantly change to lower values at higher Na+ concentration. And in [84] upon addition of 0.122 M NaCl, the radius of gyration (RG) decreased substantially, which indicates that p30 delta adopts a more compact structure. When 4 mM Mg2+ was added to native supercoiled p30 delta in 0.1 M NaCl, Rg decreased.

Using Polymethacrylate monoliths,[85], size evaluations are described on a model plasmid, consisting of 4.9 kbp, Under physiological conditions, a 45 nm radius was evaluated. But the pore size distributions in these samples (see below) are broad: as such, the changes in the median pore diameter measured by mercury intrusion porosimetry reflect general shifts in the position of the pore distribution envelope, rather than the position of a well-defined, sharp peak [86](in http://www.liv.ac.uk/~aicooper/AKH_monolith.pdf).In [87] it was found for covalently closed supercoiled ColE1-plasmid DNA in 0.2 M NaCl, 0.002 M NaPO4 pH 7.0,0.002 M EDTA, a gyration radius about 100 nm but with EDTA meaning with no divalent ions but in 0.2 M NaCl. Finally, in [88] for pGem1a plasmids (3730 base pairs) in the relaxed circular (nicked) and supercoiled forms, RG = 90 +/- 3 nm,, and RG = 82 +/- 2.5 nm were obtained.

As a conclusion, a large distribution of gyration radii is described in the literature but, they are all larger that the compact form reported by Krassowska and that she used to predict the need of "electropores" of about 10 nm for DNA translocation during the pulse. Even larger sizes are requested with linearized form, that are known to be effective for expression after their electrotransfer [39]. For a closed form (highly effective for expression after the electrotransfer), in a condensed form under physiological conditions (Na> 20 mM, MgCl2 about 1 mM), the diameter of the structures supporting a free transmembrane transfer needs to be at least 100 nm.

Simulations give shapes of supercoiled closed form of PDNA (7 kb) [89]

As NaCl concentration decreases, the superhelix becomes less regular and more compact. In the presence of just 10 mM $MgCl_2$, supercoiled DNA adopts essentially the same set of conformations as in moderate to high concentrations of NaCl.

The size of a supercoiled plasmid is difficult to access. As shown in [89], the bulk size is large. This explains why many reports are giving data around 100 nm. But in fact, it is not a sphere (a coil) but a rather elongated thread-like shape that is more relevant. The DNA topology is described quantitatively by the twist of double helix and by the number of times the helix crosses over on itself (plectoneme). Plectonemic structures are typically formed by bacterial plasmids. Then in one direction we got a cross section close to the 20 nm used by Krassowska. A larger value is indeed observed under the low salt (Mg free) solution [90, 91].

The final conclusion is that the general conformation of pDNA used for electrotransfection is "complex" and do not support the model used for the sliding model. The lack of knowledge on the theoretical processes supporting the transmembrane transport brings the need of a rather empirical approach in the optimization of the technology for gene therapy.[92].

5.2. Smaller plasmids are more effective

The basic protocols are using Naked plasmid DNA under a Double strand closed form. No advantage is brought by preparing the linear form by restriction enzymes digestion.

As size controls the efficiency of transfer, minicircle forms (MC) are more efficient [93]. Minicircle DNA lacks the bacterial backbone sequence consisting of an antibiotic resistance gene, an origin of replication, and inflammatory sequences intrinsic to bacterial DNA that represent a potential risk for safe clinical application and reduce gene transfer rates as well as transgene expression. Expression following electrotransfer is improved with MC constructs over full-length plasmid (same promoter, same coding cassette) with different reporter genes. This great efficiency of MC was correlated to more efficient vector uptake by cells. Nevertheless, one should keep in mind that huge pDNA have been transferred and that decreasing the size of the plasmid is just bringing improvement but is not needed [94].

5.3. Field effect on pDNA conformation

Under electrotransfection protocols, besides the ionic content of the buffer that can be easily adjusted under in vitro protocols (but remains poorly controlled for gene therapy) [95], a critical parameter is present. An electric field is present that may affect the conformation of the pDNA.

Concerning DNA in electric fields, conflicting observations and predictions are present in the literature. Low DC field do not greatly perturb the conformation of large DNA. In [96], it is reported that larger fields give rise to chain orientation and stretching. This is in agreement with a simulation [97]. In fact, at high concentrations, strong intermolecular aggregation was observed even under 100V/cm [98, 99]. This can be considered as an explanation for the formation of the stable spots that we observed as an early stage in the multistep process.

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6. Physical controls in optimizing the protocols

6.1. Electrical pulse parameters

Cell electropermeabilization must occur and an efficient electrophoretic accumulation of pDNA must be applied. This means that the field strength must be larger than a critical value (permeabilizing threshold at the level of the target in the tissue). Again the advantage of a targeted effect in tissues is present. But a modulation of transfer in this well defined volume by the cumulated pulse duration is present[100, 101].

The pulse duration can be short (0.1 ms) but longer pulses are more efficient as they are associated to a longer and therefore more efficient electrophoresis of the pDNA [102, 104]. The electrical parameters must be chosen to preserve the cell viability.

A double pulse method (a short high voltage pulse followed after a short delay by a long low voltage one)(HV LV) was therefore described [105]. The electrophoretic drift can be delayed from the permeabilizing pulse. This protocol is better to preserve the viability.

A destructive Joule effect can be present bringing limits in the parameters of the protocol. Intra pulse delay choice in a train of pulses can help to reduce this damaging effect by allowing a inter-pulse cooling of the pulsed sample.

6.2. Electrodes

Getting an optimized field distribution of the field intensities at the level of the target in the tissue is controlled by the geometry of the electrodes [106].

Most trials are performed by using needle electrodes that are penetrating inside the target tissues. Many designs have been reported (number of needles, diameters, distances, number, depth of penetration (see [107]). One major concern with these systems (that allow a deep penetration of the field) is the damaging effects of the electrodes (not only due to the perforation of the tissue but linked to the local effects at the electrode surface (local heating [108], electrochemical reactions [109]). Contact electrodes appear less destructive as the skin to electrodes contact is due to a conductive gel [110] but the field penetration remains rather limited

7. Cellular responses and controls

7.1. Reactive Oxygen Species (ROS)

Cellular responses are present under electrotransfection. The field induced membrane reorganization is a stress for the molecular assembly. A defense mechanism is present as shown by the generation of reactive oxygen species at the surface of the permeabilized cell [111-113]. ROS are highly destructive for DNA and reduce the number of copies that remains intact and therefore effective for expression. Protective effects are brought by the addition of anti-

oxydants as long as they are not interfering with the transport.[114, 115]. There is a need to find biocompatible additive to reduce the ROS generation. Co-block polymers appear as a promising pathway.It was shown that postshock poloxamer administration reduced tissue inflammation and damage in comparison with dextran-treated or control tissues [116].

7.2. Extracellular matrix (ECM)

Uniform DNA distribution in tumors is a prerequisite step for an homogeneous transfection efficiency in solid tumors. This is of course valid for other target tissues (skin, muscles). The interstitial space is a rate limiting physiological barrier to non-viral gene delivery. External pulsed electric fields have been proposed to increase DNA transport in the interstitium, thereby improving non-viral gene delivery. The characteristic electromobility behavior, under most electrotransfection pulsing conditions, consisted of three distinct phases: stretching, reptation, and relaxation. Electromobility depended strongly on the field magnitude, pulse duration, but a decisive role is played by the pore size of the fibrous matrix (the extracellular matrix in tumors) through which the DNA migrated [117, 118]. The intratumoral field, which determines the efficiency of electric field-mediated gene delivery, can differ significantly from the applied field at the surface of the tumor.[119]. This local field is under the control of the geometry of the electrodes as described above. The field strengths in tumor tissues were significantly lower (down to 50%) than the applied field due to the multicellular organization. But when the external field was uniform (plate parallel electrodes) the electric fields in the center region of tumors were macroscopically uniform on ex Vivo slices.

Indeed tumor histological properties strongly affected transfection efficiency. Soft tumors with larger spherical cells, low proteoglycan and collagen content, and low cell density are more effectively transfected than rigid tumors with high proteoglycan and collagen content, small spindle-shaped cells and high cell density [120].

Electrotransfection in tissue can be improved by modulation of the extracellular matrix, using collagenase and/or hyaluronidase in tumors [121] as well as in muscles, a major target organ for DNA vaccination, a great topic for gene therapy [122].

8. Conclusion

Even if our knowledge on the molecular mechanisms governing the transfer of pDNA due to the delivery of pulsed electric field remains limited, it gives recommandations for an optimal choice of the protocols.

Pulse generators should provide the largest flexibility in the choice of the electrical parameters (voltage, duration, delay, number, current intensity, sharpness of the pulse onset) and offer an internal monitoring of the delivered pulse. Very few products on the market meet these specifications.

The local field on the tissue target is a complex function of the choice of the electrodes and on the electrical changes of the tissue due to the electrical treatment. This last parameter is highly

dynamic and takes place during the pulse delivery. This remains under technical investigations by using simulations in electrical engineering [123, 124]. This time dependence of the electrical properties of the pulse tissue is an important parameter for the proper choice of the sequence of electrical pulses that must be delivered [125].

But clearly the biotechnological contributions cannot be neglected. Optimization in the plasmid constructs is strongly needed. The use of minicircles is promising to get a better transfer. But a key problem remains the design of the promoter that is shown to be an active partner in the cytoplasmic traffic to the nuclear volume.

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References

[1] Wolff, J. A, Malone, R. W, Williams, P, Chong, W, Acsadi, G, & Jani, A. Felgner PL Direct gene transfer into mouse muscle in vivo. Science. (1990). 247(4949 Pt 1), 1465-8.

[2] Neumann, E, Shaefer-ridder, M, & Wang, Y. Hofschneider PH Gene transfer into mouse lyoma cells by electroporation in high electric fields. EMBO J (1982). , 1, 841-845.

[3] Heller, R, Jaroszeski, M, Atkin, A, Moradpour, D, Gilbert, R, Wands, J, & Nicolau, C. In vivo gene electroinjection and expression in rat liver.FEBS Lett. (1996). , 389(3), 225-8.

[4] Satkauskas, S, Ruzgys, P, & Venslauskas, M. S. Towards the mechanisms for efficient gene transfer into cells and tissues by means of cell electroporation. Expert Opin Biol Ther. (2012). , 12(3), 275-86.

[5] Gothelf, A, & Gehl, J. Gene electrotransfer to skin; review of existing literature and clinical perspectives.Curr Gene Ther. (2010). , 10(4), 287-99.

[6] Kee, S. T, & Gehl, J. Lee EW Editors, Clinical aspects of Electroporation, Springer (New York), (2011).

[7] Andre, F. M, & Mir, L. M. Nucleic acids electrotransfer in vivo: mechanisms and practical aspects.Curr Gene Ther. (2010). , 10(4), 267-80.

[8] Hojman, P. Basic principles and clinical advancements of muscle electrotransfer. Curr Gene Ther. (2010). , 10(2), 128-38.

[9] Cemazar, M. Sersa G Electrotransfer of therapeutic molecules into tissues.Curr Opin Mol Ther. (2007). , 9(6), 554-62.

[10] Daud, A. I, Deconti, R. C, Andrews, S, Urbas, P, Riker, A. I, Sondak, V. K, Munster, P. N, Sullivan, D. M, Ugen, K. E, Messina, J. L, & Heller, R. Phase I trial of interleukin-12 plasmid electroporation in patients with metastatic melanoma. J Clin Oncol. (2008). , 26(36), 5896-903.

[11] Eigeldinger-berthou, S, Buntschu, P, Flück, M, Frobert, A, Ferrié, C, Carrel, T. P, Tevaearai, H. T, & Kadner, A. Electric pulses augment reporter gene expression in the beating heart. J Gene Med. (2012). doi:jgm.2603., 14(3), 191-203.

[12] Ayuni, E. L, Gazdhar, A, Giraud, M. N, Kadner, A, Gugger, M, Cecchini, M, Caus, T, Carrel, T. P, Schmid, R. A, & Tevaearai, H. T. In vivo electroporation mediated gene delivery to the beating heart. PLoS One. (2010). e14467.

[13] Hargrave, B, & Downey, H. Strange R Jr, Murray L, Cinnamond C, Lundberg C, Israel A, Chen YJ, Marshall W Jr, Heller R Electroporation-mediated gene transfer directly to the swine heart.. Gene Ther. (2012). doi:gt.2012.15.

[14] Neumann, E, Sowers, A. E, & Jordan, C. A. Electroporation and electrofusion in cell biology. York:Plenum press; (1989).

[15] Teissié, J, & Golzio, M. Rols MP Mechanisms of cell membrane electropermeabilization: a minireview of our present (lack of?) knowledge. Biochim Biophys Acta (2005). , 1724, 270-280.

[16] Orlowski, S. Mir LM Cell electropermeabilization: a new tool for biochemical and pharmacological studies. Biochim. Biophys Acta (1993). , 1154, 51-63.

[17] Eynard, N, Rols, M. P, Ganeva, V, Galutzov, B, & Sabri, N. Teissie J Electrotransformation pathways of prokaryotic and eukaryotic cells: recents developments. Bioelectrochem Bioenerg (1997). , 44, 103-10.

[18] Li, J, & Lin, H. Numerical simulation of molecular uptake via electroporation Bioelectrochemistry, (2011). , 82(1), 10-21.

[19] Pucihar, G, Kotnik, T, Miklavcic, D, & Teissié, J. Kinetics of transmembrane transport of small molecules into electropermeabilized cells. Biophys. J. (2008). , 95, 2837-2848.

[20] Mir, L. M, Roth, C, & Orlowski, S. Quintin Colonna F, Fradelizi D, et al. Systemic antitumor effects of electrochemotherapy combined with histocompatible cells secreting interleukin-2. J Immunother Emphasis Tumor Immunol (1995). , 17, 30-38.

[21] Sersa, G, Miklavcic, D, Cemazar, M, Rudolf, Z, & Pucihar, G. Snoj M Electrochemotherapy in treatment of tumours. Eur J Surg Oncol (2008). , 34, 232-240.

[22] Mir, L. M, Gehl, J, Sersa, G, Collins, C. G, Garbay, J. R, Billard, V, et al. Standard operating procedures of the electrochemotherapy: Instructions for the use of bleomycin or cisplatin administrated either systemically or locally and electric pulses delivered by the cliniporatorTM by means of invasive or non-invasive electrodes. Eur J Cancer (2006). suppl 4):14-25

[23] Mir, L. M, Glass, L. F, Sersa, G, Teissie, J, Domenge, C, Miklavcic, D, et al. Effective treatment of cutaneaous and subcutaneaous malignant tumours by electrochemotherapy. Br J Cancer (1998). , 77, 2336-2342.

[24] Testori, A, Rossi, C. R, & Tosti, G. Utility of electrochemotherapy in melanoma treatment. Curr Opin Oncol. (2012). , 24(2), 155-61.

[25] Campana, L. G, Mocellin, S, Basso, M, Puccetti, O, De Salvo, G. L, Chiarion-sileni, V, Vecchiato, A, Corti, L, Rossi, C. R, & Nitti, D. Bleomycin-based electrochemotherapy: clinical outcome from a single institution's experience with 52 patients.Ann Surg Oncol. (2009). , 16(1), 191-9.

[26] Hampton T Electric Pulses Help With ChemotherapyMay Open New Paths for Other Agents,JAMA, (2011). , 305, 549-51.

[27] Weaver, J. C. Chizmadzhev YA Theory of electroporation: A review. Bioelectrochem Bioenerg (1996). , 41, 135-160.

[28] Aihara, H. Miyazaki JI Gene transfer into muscle by electroporation in vivo. Nature Biotechnol (1998). , 16, 867-870.

[29] Mir, L. M, Bureau, M. F, Gehl, J, Rangara, R, Rouy, D, Caillaud, J. M, Delaere, P, Branellec, D, & Schwartz, B. Scherman D High-efficiency gene transfer into skeletal muscle mediated by electric pulses. Proc Natl Acad Sci USA (1999). , 96, 4262-4267.

[30] Liu, F. Huang L Electric gene transfer to the liver following systemic administration of plasmid DNA. Gene Ther (2002). , 9, 1116-1119.

[31] Titomirov, A. V, & Sukharev, S. Kistanova E In vivo electroporation and stable transformation of skin cells of newborn mice by plasmid DNA. Biochim Biophys Acta Report (1991). , 1088, 131-134.

[32] Vandermeulen, G, Staes, E, Vanderhaeghen, M. L, Bureau, M. F, & Scherman, D. Préat V Optimisation of intradermal DNA electrotransfer for immunisation. J Control Res (2007). , 124, 81-87.

[33] Kaufman, C. D, Geiger, R. C, & Dean, D. A. Electroporation- and mechanical ventilation-mediated gene transfer to the lung. Gene therapy. (2010). , 17(9), 1098-104.

[34] Rols, M. P, Delteil, C, Golzio, M, Dumond, P, & Cros, S. Teissie J In vivo electrically mediated protein and gene transfer in murine melanoma. Nat Biotechnol (1998). , 16, 168-171.

[35] Bettan, M, Ivanov, M. A, Mir, L. M, Boissiere, F, & Delaere, P. Scherman D Efficient DNA electrotransfer into tumors Bioelectrochemistry (2000).

[36] Tevz, G, Pavlin, D, Kamensek, U, Kranjc, S, Mesojednik, S, Coer, A, Sersa, G, & Cemazar, M. Gene electrotransfer into murine skeletal muscle: a systematic analysis of parameters for long-term gene expression Technol Cancer Res Treat. (2008). , 7(2), 91-101.

[37] De Gennes, P. G. Passive entry of a DNA molecule into a small pore Proc. Natl. Acad. Sci. USA (1999). , 96, 7262-7264.

[38] Yu, M, Tan, W, & Lin, H. A stochastic model for DNA translocation through an electropore BBA Biomembranes (2012). , 1818(11), 2494-2501.

[39] Escoffre, J. M, Nikolova, B, Mallet, L, Henri, J, Favard, C, Golzio, M, Teissié, J, & Tsoneva, I. Rols MP New insights in the gene electrotransfer process: Evidence for the involvement of the plasmid DNA topology. Curr Gene Ther. (2012). Epub ahead of print]

[40] Smith, K. C, & Neu, J. C. Krassowska W Model of creation and evolution of stables electropores for DNA delivery. Biophys J (2004). , 86, 2813-2826.

[41] Krassowska, W. Filev PD Modeling electroporation in a single cell. Biophys J (2007). , 92, 404-417.

[42] Neu, J. C. Krassowska W Modeling postshock evolution of large electropores. Phys Rev E Stat Nonlin Soft Matter Phys (2003).

[43] Sukharev, S. I, & Titomirov, A. V. Klenchin VA Electrically induced DNA transfer into cells. Electroporation in vivo. In Gene Therapeutics: Methods and Applications of Direct Gene Transfer. JA Wolff, editor. Birkhäuser, Boston, MA. 210-232,(1994).

[44] Bier, M, Hammer, S. M, & Canaday, D. J. Lee RC Kinetics of resealing for transient electropores in isolated mammalian skeletal muscle cells. Bioelectromagnetics (1999). , 20, 194-201.

[45] Golzio, M, Mora, M. P, Raynaud, C, Delteil, C, & Teissie, J. Rols MP Control by osmotic pressure of voltage-induced permeabilization and gene transfer in mammalian cells. Biophys J (1998). , 74, 3015-3022.

[46] Satkauskas, S, Bureau, M. F, Puc, M, Mahfoudi, A, Scherman, D, & Miklavcic, D. Mir LM Mechanisms of in vivo DNA electrotransfer: respective contributions of cell electropermeabilization and DNA electrophoresis. Mol Ther (2002). , 5, 133-140.

[47] Tekle, E, & Astumian, R. D. Chock PB Electroporation by using bipolar oscillating electric field: an improved method for DNA transfection of NIH 3T3 cells. Proc Natl Acad Sci USA (1991). , 88, 4230-4234.

[48] Freeman, S. A, & Wang, M. A. Weaver JC Theory of electroporation of planar bilayer membranes: predictions of the aqueous area, change in capacitance, and pore-pore separation. Biophys J (1994). , 67, 42-56.

[49] Joshi, R. P. Schoenbach KH Electroporation dynamics in biological cells subjected to ultrafast electrical pulses: a numerical simulation study. Phys Rev E (2000). , 62, 1025-1033.

[50] Kakorin, S, & Neumann, E. Ionic conductivity of electroporated lipid bilayer membranes. Bioelectrochem (2002). , 56, 163-166.

[51] Neumann, E, Kakorin, S, Tsoneva, I, & Nikolova, B. Tomov T Calcium-mediated DNA adsorption to yeast cells and kinetics of cell transformation by electroporation. Biophys J (1996). , 71, 868-877.

[52] Rols, M. P. Teissie J Electropermeabilization of mammalian cells to macromolecules: control by pulse duration. Biophys J (1998). , 75, 1415-1423.

[53] Rols, M. P, & Delteil, C. Teissie J Control by ATP and ADP of voltage-induced mammalian-cell-membrane permeabilization, gene transfer and resulting expression. Eur J Biochem (1998). , 254, 382-388.

[54] Sukharev, S. I, Klenchin, V. A, Serov, S. M, & Chernomordik, L. V. Chizmadzhev, Yu A Electroporation and electrophoretic DNA transfer into cells. The effect of DNA interaction with electropores. Biophys J (1992). , 63, 1320-1327.

[55] Neumann, E, & Kakorin, S. Toensing K Fundamentals of electroporative delivery of drugs and genes. Bioelectrochem Bioenerg (1999). , 48, 3-16.

[56] Hristova, N. I, & Tsoneva, I. Neumann E Sphingosine-mediated electroporative DNA transfer through lipid bilayers. FEBS Lett (1997). , 415, 81-86.

[57] Spassova, M, Tsoneva, I, Petrov, A. G, & Petkova, J. I. Neumann E Dip patch clamp currents suggest electrodiffusive transport of the polyelectrolyte DNA through lipid bilayers. Biophys Chem (1994). , 52, 267-274.

[58] Winterbourne, D. J, Thomas, S, Hermon-taylor, J, & Hussain, I. Johnstone AP Electric-shock-mediated transfection of cells. Biochem J (1988). , 251, 427-434.

[59] Klenchin, V. A, Sukharev, S. I, Serov, S. M, & Chernomordik, L. V. Chizmadzev YuA Electrically induced DNA uptake by cells is a fast process involving DNA electrophoresis. Biophys J (1991). , 60, 804-811.

[60] Tekle, E, & Astumian, R. D. Chock PB Selective and asymmetric molecular transport across electroporated cell membranes. Proc Natl Acad Sci USA (1994). , 91, 11512-11516.

[61] Hui SW Effects of pulse length and strength on electroporation efficiencyMethods Mol Biol (1995). , 55, 29-40.

[62] Wu, M. Yuan F Membrane Binding of Plasmid DNA and Endocytic Pathways Are Involved in Electrotransfection of Mammalian Cells. PLoS ONE (2011). e20923. doi: 10.1371/journal.pone.0020923

[63] Tarek M Membrane electroporation: a molecular dynamics simulationBiophys J (2005). , 88, 4015-4053.

[64] Bandyopadhyay, S, Tarek, M, & Klein, M. L. (1999). Molecular dynamics study of lipid-DNA complexes. J Phys Chem B 1999; , 103, 10075-10080.

[65] Lin, R, & Chang, D. C. Lee YK Single-cell electroendocytosis on a micro chip using in situ fluorescence microscopy. Biomed Microdevices. (2011). , 13(6), 1063-73.

[66] Rosazza, C, Phez, E, Escoffre, J. M, Cézanne, L, Zumbusch, A, & Rols, M. P. Cholesterol implications in plasmid DNA electrotransfer: Evidence for the involvement of endocytotic pathways. Int J Pharm. (2012). , 423(1), 134-43.

[67] Golzio, M, & Teissié, J. Rols MP Direct visualization at the single-cell level of electrically mediated gene delivery. Proc Natl Acad Sci USA (2002). , 99, 1292-1297.

[68] Pavlin, M, & Pucihar, G. Kandušer M The role of electrically stimulated endocytosis in gene electrotransferBioelectrochemistry (2012). , 83, 38-45.

[69] Pavlin, M, Flisar, K, & Kandušer, M. The role of electrophoresis in gene electrotransfer. J. Membrane Biol. (2010). , 236, 75-79.

[70] Escoffre, J. M, Portet, T, Favard, C, Teissié, J, Dean, D. S, & Rols, M. P. Electromediated formation of DNA complexes with cell membranes and its consequences for gene delivery.Biochim Biophys Acta. (2011). , 1808(6), 1538-43.

[71] Faurie, C, Rebersek, M, Golzio, M, Kanduser, M, Escoffre, J. M, Pavlin, M, Teissie, J, & Miklavcic, D. Rols MP Electro-mediated gene transfer and expression are controlled by the life-time of DNA/membrane complex formation. J Gene Med. (2010). , 12(1), 117-25.

[72] Van Der Aa, M. A, Mastrobattista, E, Oosting, R. S, Hennik, W. E, Koning, G. A, & Crommelin, D. J. The nuclear pore complex: the gateway to successful nonviral gene delivery. Pharm Res (2006). , 23, 447-459.

[73] Van Der Aa, M. A, Mastrobattista, E, Oosting, R. S, Hennink, W. E, & Koning, G. A. Crommelin DJ The nuclear pore complex: the gateway to successful nonviral gene delivery. Adv Drug Deliv Rev (2007). , 59, 698-717.

[74] Pouton, C. W, Wagstaff, K. M, Roth, D. M, & Moseley, G. W. Jans DA Targeted delivery to the nucleus. Adv Drug Deliv Rev (2007). , 59, 698-717.

[75] Bellard, E. Teissie J Double-Pulse Approach of Electrogenotherapy: An Analysis at the Single Cell Level- IEEE Trans Plasma Sci, (2009)., 37(4), 538-44.

[76] Bellard, E. Teissié J Double pulse approach of electropulsation: a fluorescence analysis of the nucleus perturbation at the single cell level IEEE Trans Dielect Electr In, (2009). , 16, 1267-1272.

[77] Esser, A. T, Smith, K. C, Gowrishankar, T. R, Vasilkoski, Z, & Weaver, J. C. Mechanisms for the intracellular manipulation of organelles by conventional electroporation. Biophys J. (2010). , 98(11), 2506-14.

[78] Rosazza, C, Escoffre, J. M, Zumbusch, A, & Rols, M. P. The actin cytoskeleton has an active role in the electrotransfer of plasmid DNA in mammalian cells. Mol Ther. (2011)., 19(5), 913-21.

[79] Badding, M. A, & Vaugham, E. E. Dean DA Transcription factor plasmid binding modulates microtubule interactions and intracellular trafficking during gene transfer Gene Therapy (2012). , 19, 338-346.

[80] Miller, A. M. Dean DA Tissue-specific and transcription factor-mediated nuclear entry of DNA. Adv Drug Deliv Rev (2009). , 61, 603-613.

[81] Lam, A. P. Dean DA Progress and prospects: nuclear import of nonviral vectors Gene Therapy (2010). , 17, 439-447.

[82] Vologodskii, A. V, & Cozzarelli, N. R. Conformational and thermodynamic properties of supercoiled DNA. Annu Rev Biophys Biomol Struct. (1994). , 23, 609-43.

[83] Hammermann, M, Brun, N, Klenin, K. V, May, R, & Tóth, K. Langowski JSalt-dependent DNA superhelix diameter studied by small angle neutron scattering measurements and Monte Carlo simulations. Biophys J. (1998). , 75(6), 3057-63.

[84] Gebe, J. A, Delrow, J. J, Heath, P. J, Fujimoto, B. S, & Stewart, D. W. Schurr JM Effects of Na+ and Mg2+ on the structures of supercoiled DNAs: comparison of simulations with experiments. J Mol Biol. (1996). , 262(2), 105-28.

[85] Zöchling, A, Hahn, R, Ahrer, K, Urthaler, J, & Jungbauer, A. Mass transfer characteristics of plasmids in monoliths. J Sep Sci. (2004)., 27(10-11), 819-27.

[86] http://wwwliv.ac.uk/~aicooper/AKH_monolith.pdf

[87] Voordouw, G, Kam, Z, & Borochov, N. Eisenberg H Isolation and physical studies of the intact supercoiled, the open circular and the linear forms of ColE1-plasmid DNA. Biophys Chem. (1978). , 8(2), 171-89.

[88] Fishman, D. M, & Patterson, G. D. Light scattering studies of supercoiled and nicked DNA. Biopolymers. (1996). , 38(4), 535-52.

[89] Rybenkov, V, & Vologodskii, A. V. Cozzarelli NR The effect of ionic conditions on the conformations of supercoiled DNA. I. sedimentation analysis1 Journal of Molecular Biology (1997). , 267, 299-311.

[90] Rybenkov, V, & Vologodskii, A. V. Cozzarelli NR The effect of ionic conditions on DNA helical repeat,effective diameter and free energy of supercoiling Nucleic Acids Research, (1997). , 25, 1412-8.

[91] Baranello, L, Levens, D, & Guptaa, A. Kouzine F The importance of being supercoiled: How DNA mechanics regulate dynamic processes Biochimica et Biophysica Acta

(BBA)- Gene Regulatory Mechanisms (2012). Chromatin in time and space, 1819, 632-638.

[92] Golzio, M, Escoffre, J. M, Portet, T, Mauroy, C, Teissié, J, Dean, D. S, & Rols, M. P. Observations of the mechanisms of electromediated DNA uptake--from vesicles to tissues. Curr Gene Ther. (2010). , 10(4), 256-66.

[93] Kobelt, D, Schleef, M, Schmeer, M, Aumann, J, & Schlag, P. M. Walther W Performance of High Quality Minicircle DNA for In Vitro and In Vivo Gene Transfer Mol Biotechnol (2012). on line DOIs12033-012-9535-6).

[94] Chabot, S, Orio, J, Schmeer, M, Schleef, M, Golzio, M, & Teissié, J. Minicircle DNA electrotransfer for efficient tissue-targeted gene delivery. Gene Ther. (2013)., 20(1), 62-8 doi:gt.2011.215

[95] Lee, M. J, Cho, S. S, Jang, H. S, Lim, Y. S, You, J. R, Park, J, Suh, H, Kim, J. A, Park, J. S, & Kim, D. K. Optimal salt concentration of vehicle for plasmid DNA enhances gene transfer mediated by electroporation. Exp Mol Med. (2002). , 34(4), 265-72.

[96] Jonsson, M, Jacobsson, U, & Takahashi, M. Nordén B Orientation of large DNA during free solution electrophoresis studied by linear dichroism J Chem Soc Faraday trans (1993), 89, 2791–2798

[97] Netz RR Nonequilibrium Unfolding of Polyelectrolyte Condensates in Electric Fields Phys Rev Lett 2003; 90:128104

[98] Magnúsdóttir, S, Isambert, H, Heller, C, & Viovy, J. L. Electrohydrodynamically induced aggregation during constant and pulsed field capillary electrophoresis of DNA. Biopolymers (1999)., 49, 385–401

[99] Mitnik, L, Heller, C, & Prost, J. Viovy JL Segregation in DNA solutions induced by electric fields. Science. (1995). , 267(5195), 219-22.

[100] Cemazar, M, Golzio, M, Sersa, G, Rols, M. P, & Teissié, J. Electrically-assisted nucleic acids delivery to tissues in vivo: where do we stand? Curr Pharm Des. (2006). , 12(29), 3817-25.

[101] Miklavcic, D, Beravs, K, Semrov, D, Cemazar, M, Demsar, F, & Sersa, G. The importance of electric field distribution for effective in vivo electroporation of tissues.Biophys J. (1998). , 74(5), 2152-8.

[102] Satkauskas, S, Bureau, M. F, Puc, M, Mahfoudi, A, Scherman, D, Miklavcic, D, & Mir, L. M. Mechanisms of in vivo DNA electrotransfer: respective contributions of cell electropermeabilization and DNA electrophoresis. Mol Ther. (2002). , 5(2), 133-40.

[103] Satkauskas, S, André, F, Bureau, M. F, Scherman, D, Miklavcic, D, & Mir, L. M. Electrophoretic component of electric pulses determines the efficacy of in vivo DNA electrotransfer. Hum Gene Ther. (2005). , 16(10), 1194-201.

[104] Cemazar, M, Golzio, M, Sersa, G, Hojman, P, Kranjc, S, Mesojednik, S, Rols, M. P, & Teissie, J. Control by pulse parameters of DNA electrotransfer into solid tumors in mice. Gene Ther. (2009). , 16(5), 635-44.

[105] André, F. M, Gehl, J, Sersa, G, Préat, V, Hojman, P, Eriksen, J, Golzio, M, Cemazar, M, Pavselj, N, Rols, M. P, Miklavcic, D, Neumann, E, Teissié, J, & Mir, L. M. Efficiency of high- and low-voltage pulse combinations for gene electrotransfer in muscle, liver, tumor, and skin.Hum Gene Ther. (2008). , 19(11), 1261-71.

[106] Gothelf, A, Mahmood, F, Dagnaes-hansen, F, & Gehl, J. Efficacy of transgene expression in porcine skin as a function of electrode choice. Bioelectrochemistry. (2011). , 82(2), 95-102.

[107] Guo, S, Donate, A, Basu, G, Lundberg, C, Heller, L, & Heller, R. Electro-gene transfer to skin using a noninvasive multielectrode array. J Control Release. (2011). , 151(3), 256-62.

[108] Davalos, R. V, Rubinsky, B, & Mir, L. M. Theoretical analysis of the thermal effects during in vivo tissue electroporation. Bioelectrochemistry. (2003)., 61, 99–107

[109] Turjanski, P, Olaiz, N, Maglietti, F, Michinski, S, Suárez, C, Molina, F. V, & Marshall, G. The role of pH fronts in reversible electroporation. PLoS One. (2011). e17303

[110] Golzio, M, Mazeres, S, & Teissie, J. Electrodes for in vivo localised subcutaneous electropulsation and associated drug and nucleic acid delivery. Expert Opin Drug Deliv. (2009). , 6(12), 1323-31.

[111] Gabriel, B, & Teissié, J. Control by electrical parameters of short- and long-term cell death resulting from electropermeabilization of Chinese hamster ovary cells.Biochim Biophys Acta. (1995). , 1266(2), 171-8.

[112] Bonnafous, P, Vernhes, M, Teissié, 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.Biochim Biophys Acta. (1999). , 1461(1), 123-34.

[113] Pakhomova, O. N, Khorokhorina, V. A, Bowman, A. M, Rodaite-riševiciene, R, Saulis, G, Xiao, S, & Pakhomov, A. G. Oxidative effects of nanosecond pulsed electric field exposure in cells and cell-free media.Arch Biochem Biophys. (2012). Aug 15. [Epub ahead of print]).

[114] Markelc, B, Tevz, G, Cemazar, M, Kranjc, S, Lavrencak, J, Zegura, B, Teissie, J, & Sersa, G. Muscle gene electrotransfer is increased by the antioxidant tempol in mice. Gene Ther. (2012). , 19(3), 312-20.

[115] Sabri, N, Pelissier, B, & Teissie, J. Ascorbate increases electrotransformation efficiency of intact maize cells. Anal Biochem. (1998). , 264(2), 284-6.

[116] Lee, R. C, River, L. P, Pan, F. S, & Ji, L. Wollmann RL Surfactant-induced sealing of electropermeabilized skeletal muscle membranes in vivo. Proc Natl Acad Sci U S A. (1992). , 89(10), 4524-8.

[117] Henshaw, J. W, Zaharoff, D. A, Mossop, B. J, & Yuan, F. A single molecule detection method for understanding mechanisms of electric field-mediated interstitial transport of genes Bioelectrochemistry. (2006). , 69(2), 248-53.

[118] Henshaw, J. W, Zaharoff, D. A, & Mossop, B. J. Yuan F Electric field-mediated transport of plasmid DNA in tumor interstitium in vivo Bioelectrochemistry (2007). , 71, 233-242.

[119] Mossop, B. J, Barr, R. C, Henshaw, J. W, & Zaharoff, D. A. YuanF Electric Fields in Tumors Exposed to External Voltage Sources: Implication for Electric Field-Mediated Drug and Gene Delivery Annals of Biomedical Engineering, (2006). C 2006):1564-1572

[120] Mesojednik, S, Pavlin, D, Sersa, G, Coer, A, Kranjc, S, Grosel, A, & Tevz, G. Cemazar M The effect of the histological properties of tumors on transfection efficiency of electrically assisted gene delivery to solid tumors in mice. Gene Ther. (2007). , 14(17), 1261-9.

[121] Cemazar, M, Golzio, M, Sersa, G, Escoffre, J. M, Coer, A, Vidic, S, & Teissie, J. Hyaluronidase and collagenase increase the transfection efficiency of gene electrotransfer in various murine tumors. Hum Gene Ther. (2012). , 23(1), 128-37.

[122] Mcmahon, J. M, Signori, E, Wells, K. E, Fazio, V. M, & Wells, D. J. Optimisation of electrotransfer of plasmid into skeletal muscle by pretreatment with hyaluronidase--increased expression with reduced muscle damage. Gene Ther. (2001). , 8(16), 1264-70.

[123] Zupanic, A, & Kos, B. Miklavcic D Treatment planning of electroporation-based medical interventions: electrochemotherapy, gene electrotransfer and irreversible electroporation. Phys Med Biol. (2012). , 57(17), 5425-40.

[124] Zupanic, A, Corovic, S, Miklavcic, D, & Pavlin, M. Numerical optimization of gene electrotransfer into muscle tissue. Biomed Eng Online. (2010)., 9():66.

[125] Cukjati, D, Batiuskaite, D, André, F, & Miklavcic, D. Mir LM Real time electroporation control for accurate and safe in vivo non-viral gene therapy. Bioelectrochemistry. (2007). , 70(2), 501-7.