Chapter 7
How Imaging Membrane and Cell Processes Involved in Electropermeabilization Can Improve Its Development in Cell Biology and in Clinics

Laure Gibot, Muriel Golzio, and Marie-Pierre Rols

Abstract Cell membranes can be transiently permeabilized under the application of electric pulses. This process, called electropermeabilization or electroporation, allows hydrophilic molecules, such as anticancer drugs and DNA, to enter into cells and tissues. The method is nowadays used in clinics to treat cancers. Vaccination and gene therapy are other fields of application of DNA electrotransfer. A description of the mechanisms can be assayed by using different complementary systems with increasing complexities (models of membranes, cells cultivated in 2D and 3D culture named spheroids, and tissues in living mice) and different microscopy tools to visualize the processes from single molecules to entire animals. Single-cell imaging experiments revealed that the uptake of molecules (nucleic acids, antitumor drugs) takes place in well-defined membrane regions and depends on their chemical and physical properties (size, charge). If small molecules freely cross the electropermeabilized membrane and have a free access to the cytoplasm, larger molecules, such as plasmid DNA, face physical barriers (plasma membrane, cytoplasm crowding, nuclear envelope) which reduce transfection efficiency and engender a complex mechanism of transfer. Gene electrotransfer indeed involves different steps that include the initial interaction with the membrane, its crossing, transport within the cytoplasm, and finally gene expression. In vivo, additional very important effects of electric pulses are present such as blood flow modifications. The full knowledge on the way molecules are transported across the electropermeabilized membranes and within tissues is mandatory to improve the efficacy and the safety of the electropermeabilization process both in cell biology and in clinics.

L. Gibot • M. Golzio • M.-P. Rols (✉)
Institut de Pharmacologie et de Biologie Structurale, Université de Toulouse, CNRS, UPS, 205 Route de Narbonne, BP 64182, F-31077 Toulouse, France
e-mail: marie-pierre.rols@ipbs.fr

J. Kulbacka, S. Satkauskas (eds.), *Transport Across Natural and Modified Biological Membranes and its Implications in Physiology and Therapy*, Advances in Anatomy, Embryology and Cell Biology 227,
DOI 10.1007/978-3-319-56895-9_7

7.1 Introduction

Electropermeabilization, also called electroporation (EP), is based on the reversible permeabilization of cell membranes, thus enabling the delivery of non-permeant or poorly permeant molecules into the cells. The use of electric field pulses to deliver therapeutic molecules including drugs, proteins, and nucleic acids in a wide range of cells and tissues has been largely developed over the last decades (Andre and Mir 2010; Yarmush et al. 2014; Rosazza et al. 2016). This physical method is nowadays used in clinics to treat cancers, a process named electrochemotherapy (ECT). ECT combines the local application of well-defined electric field pulses following the local or systemic injection of antitumor drugs such as bleomycin and cisplatin (Mir et al. 1998; Escoffre and Rols 2012). Although ECT is used in routine clinical practice for treatment of subcutaneous tumors in more than 140 clinics and hospitals throughout Europe, mechanisms involved are still not fully understood. Reversible permeabilization of the cell membrane is the basic mechanism of the antitumor effectiveness. But the fact that ECT fairly preserves healthy tissues is not completely explained, as well as the effects of EP on blood circulation and vessels permeability.

Besides ECT, vaccination and oncology gene therapy are also major fields of application of DNA electrotransfer (Chiarella et al. 2010; Sersa et al. 2015). Translation of preclinical studies into clinical trials in human and veterinary oncology has started (Cemazar et al. 2010; Heller and Heller 2010). The first phase I dose escalation trial of electroporation of plasmid encoding for interleukin 12 has been carried out in patients with metastatic melanoma and has shown encouraging results (Daud et al. 2008). But the safe and efficient use of this physical method for clinical purposes requires the knowledge of the mechanisms underlying the electropermeabilization phenomena. Despite the fact that the pioneering work on plasmid DNA electrotransfer in cells was initiated 35 years ago (Neumann et al. 1982), many of the mechanisms underlying DNA electrotransfer remain to be elucidated (Teissie et al. 2005) as the way tissues respond to the electric pulses (Kamensek et al. 2016). Even if in vitro electrotransfer is efficient in almost all cell lines, in vivo gene delivery and expression in tumors are usually not (Rols et al. 1998). It is still mandatory, for increasing gene transfer and expression, to increase our knowledge of the different processes occurring both in vitro and in vivo.

This review focuses on (1) what it is (still not) known about the processes of transport of molecules across the membranes submitted to electric pulses and (2) how this knowledge helps to define electric parameters for improving the efficacy and safety of the electropermeabilization process both in cell biology and in clinics. It will describe key experiments obtained by using convenient imaging tools to directly visualize the different processes of transport and this on different experimental models with increasing complexities from simple model of membranes to tissues (Fig. 7.1).

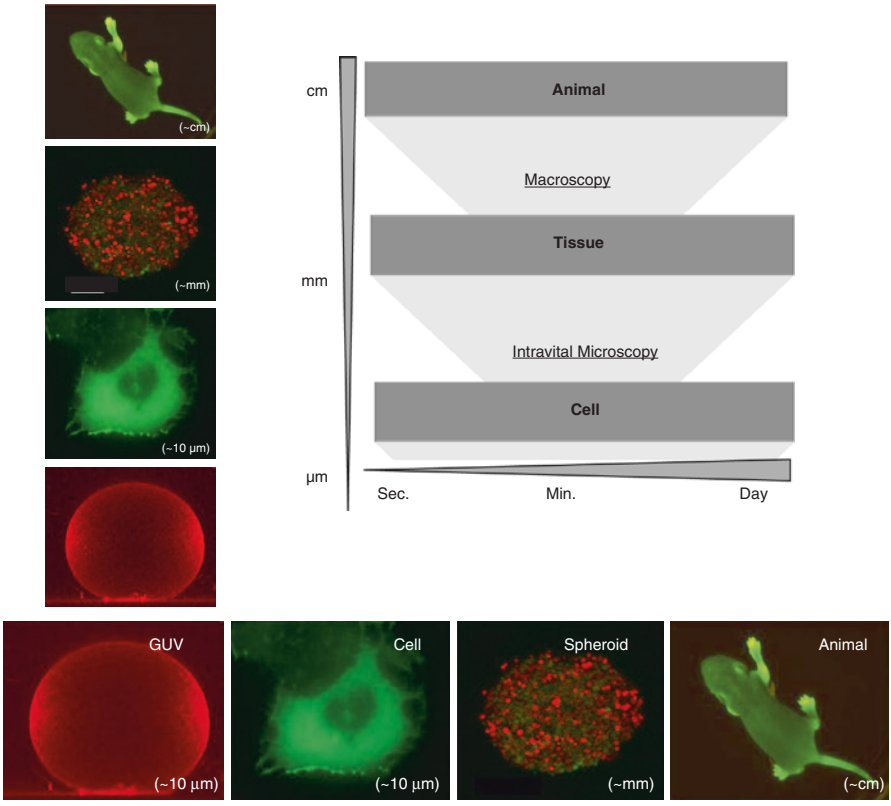

Fig. 7.1 Models of increasing complexities and imaging tools used to address electroporation processes

7.2 Imaging Membrane Processes

7.2.1 Membrane Electropermeabilization

Giant unilamellar vesicles (GUVs) are useful to study the effect of permeabilizing electric fields in simple membrane models. GUVs represent a convenient way to study membrane properties such as lipid bilayer composition and membrane tension. They offer the possibility to study and visualize membrane processes due to their cell-like size in absence of any constraint due to cytoskeleton. Experiments performed by phase contrast and fluorescence microscopies as well as by CARS spectroscopy showed a decrease in vesicle radius which is interpreted as being due to lipid loss during the permeabilization process (Mauroy et al. 2012). Three mechanisms responsible for lipid loss were directly observed, pore formation, vesicle

formation, and tubule formation, which may be involved in molecule uptake (Portet et al. 2009). GUVs are also a good model to study the mechanisms of electrofusion, with a direct interest to their use as vehicles to deliver molecules (Mauroy et al. 2012). However, GUV cannot be used to study the whole process involved in the transport of large molecules such as plasmid DNA where nuclear expression is required. Indeed, a direct transfer of DNA into the GUVs took place during the application of the electric pulses (Portet et al. 2011), which is contradictory with what was observed in unique cell context.

The use of video microscopy allowed visualization of the permeabilization phenomenon at the single-cell level in cells grown on Petri dishes. Propidium iodide is a very convenient molecule that allows to monitor membrane electropermeabilization. Its size is at the same range of order of a large variety of therapeutic drugs. Its uptake into the cytoplasm is a fast process that is induced during electric pulse delivery and that can be detected seconds after the application of electric pulses. Exchange across the permeabilized membrane is not homogeneous on the whole cell membrane. It occurs at the sides of the cells facing the electrodes in an asymmetrical way where it is more pronounced at the anode-facing side of the cells than at the cathode, i.e., in the hyperpolarized area than in the depolarized area, which is in agreement with theoretical considerations (Fig. 7.2) (Teissie et al. 2005).

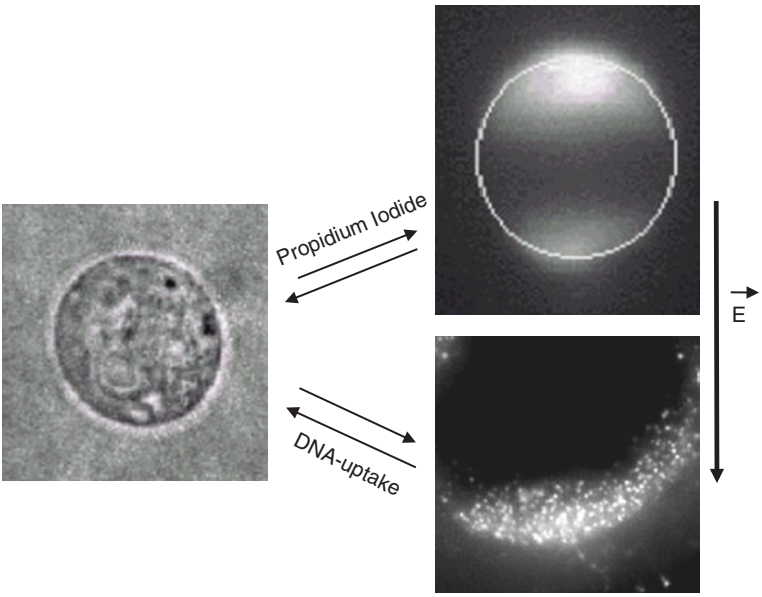

Fig. 7.2 Different steps involved in membrane electropermeabilization and gene electrotransfer as directly visualized under a microscope a few seconds following pulses delivery. Before pulse application, the membrane is used as a barrier that prevents the passage of small hydrophilic molecules such as propidium iodide and large and charged molecules such as plasmid DNA. Electric pulse application induces the permeabilization of the cell membrane facing the two electrodes and DNA interaction facing the cathode

Electropermeabilization can be described as a three-step process in respect with electric field: (1) before electropulsation, the plasma membrane acts as a physical barrier that prevents the free exchange of hydrophilic molecules between the cell cytoplasm and external medium; (2) during electropulsation, the transmembrane potential increases which induces the formation of transient permeable structures facing the electrodes and allows the exchange of molecules; and (3) after electropulsation, membrane resealing occurs within minutes.

A direct transfer into the cell cytoplasm of the negatively charged small molecules such as siRNA is observed on the side facing the cathode. When added after electropulsation, siRNA do not penetrate the cells. Therefore, electric field acts on both the permeabilization of the membrane and on the electrophoretic drag of the charged molecules from the bulk into the cytoplasm. The mechanism involved is clearly specific for the physicochemical properties of the electrotransferred molecule (Paganin-Gioanni et al. 2011; Golzio and Teissie 2014).

Progress in the knowledge of the involved mechanisms at the molecular level is still a biophysical challenge. Once again, fluorescence microscopy helped to go deeper into the elucidation of the mechanisms. The electric pulses induced the formation of long-lived permeant structures and resulted in a rapid phospholipid flip-flop within less than 1 s and were exclusively restricted to the regions of the permeabilized membrane. These results could support the existence of direct interactions between the movement of membrane zwitterionic phospholipids and the electric field (Escoffre et al. 2014). In addition, experiments on lateral mobility of proteins showed that electropermeabilization affects the lateral mobility of membrane protein, a result that suggests that 10–20% of the membrane surface is occupied by defects or pores and that these structures propagate rapidly over the cell surface (Escoffre et al. 2014).

It is also possible to take advantage of atomic force microscopy to directly visualize the consequences of electropermeabilization (without using any fluorescent molecule) and to locally measure the membrane elasticity. The transient rippling of membrane surface has been visualized which was associated to a decrease in membrane elasticity. These results obtained both on fixed and living cells give evidence of an inner effect affecting the entire cell surface which may be related to cytoskeleton destabilization and not only the area where transfer of molecules takes place (Chopinet et al. 2013).

7.2.2 Electrotransfer of DNA Molecules

Single-cell microscopy and fluorescent plasmids can be used to monitor the different steps of gene electrotransfer (GET) (Golzio et al. 2002). As in the case of siRNA, DNA molecules, which are negatively charged, migrate electrophoretically. Under electric fields that are too small to permeabilize the membrane, the DNA flows around the cell. Beyond a critical field value, above which cell permeabilization occurs, the DNA interacts with the electropermeabilized plasma membrane. This interaction

only occurs at the pole of the cell opposite the cathode, and this demonstrates the importance of electrophoretic forces in the initial phase of the DNA/membrane interaction. DNA/membrane interaction is correlated to the formation of "microdomains" whose dimensions lie between 0.1 and 0.5 μm (Fig. 7.2). DNA electrotransfer can be described as a multistep process: the negatively charged DNA migrates electrophoretically toward the plasma membrane on the cathode side where it accumulates. This interaction, which is observed for several minutes, lasts much longer than the duration of the electric field pulses. Translocation of the plasmid from the plasma membrane to the cytoplasm and its subsequent passage toward the nuclear envelope take place with a kinetics ranging from minutes to hours. When plasmids have reached the nuclei, gene expression can take place, and this can be detected up to several days in the case of dividing cells or weeks in some tissues such as muscles.

The dynamic of this process has been poorly understood because direct observations have been limited to time scales that exceed several seconds. The use of a camera with a temporal resolution of 2 ms allowed the visualization of the DNA/membrane interaction process during pulse application. DNA molecules interact with the membrane during the application of the pulse. At the beginning of the pulse application, plasmid complexes or aggregates appear at specific sites on the cell membrane. The formation of plasmid complexes at fixed sites suggests that membrane domains may be responsible for DNA uptake and their lack of mobility could be due to their interaction with the actin cytoskeleton. FRAP measurements show that the positions of these sites are remarkably immobile during the application of further pluses. A theoretical model is proposed to explain the appearance of distinct interaction sites, the quantitative increase in DNA, and also their immobility leading to a tentative explanation for the success of electromediated gene delivery (Escoffre et al. 2011).

DNA/membrane interaction and gene expression depend on electric pulse polarity, repetition frequency, and duration. Both are affected by reversing the polarity and by increasing the repetition frequency or the duration of pulses. The results revealed the existence of two classes of DNA/membrane interaction: (1) a metastable DNA/membrane complex from which DNA can leave and return to external medium and (2) a stable DNA/membrane complex, where DNA cannot be removed, even by applying electric pulses of reversed polarity. Only DNA belonging to the second class leads to effective gene expression. The lifetime of DNA/membrane complex formation is in the order of 1 s and has to be taken into account to improve protocols of electromediated gene delivery (Faurie et al. 2010).

Even if the first stage of gene electrotransfection, i.e., migration of plasmid DNA toward the electropermeabilised plasma membrane and its interaction with it, becomes understood, it is not totally possible today to give guidelines to improve gene electrotransfer. Successful expression of the plasmid depends on its subsequent migration into the cell. Therefore, the intracellular diffusional properties of plasmid DNA, as well as its metabolic instability and nuclear translocation, represent other cell-limiting factors that must be taken into account. In the conditions induced during electropermeabilization, the time a plasmid DNA takes to reach the nuclei is significantly longer than the time needed for a small molecule (Rosazza et al. 2011). Single-particle tracking experiments of individual DNA aggregates in living cells allowed further elucidation of the mechanism of DNA transfer. Active transport was visualized over long distances and has been shown to be related to the

cellular microtubule network (Rosazza et al. 2013). In addition, the use of inhibitors of endocytosis and endosomal markers showed that, during active transport, DNA is routed through endosomal compartments.

Clear limits of efficient gene expression using electric pulses are therefore due to the passage of DNA molecules through the plasma membrane and to the cytoplasmic crowding and transfer through the nuclear envelope. A key challenge for electromediated gene therapy is to pinpoint the rate-limiting steps in this complex process and to find strategies to overcome these obstacles. One of the possible strategies to enhance DNA uptake into cells and to induce effects that primarily affect intracellular structures and functions is to use short (10–300 ns) but high pulse (up to 300 kV/cm) (Beebe et al. 2003). The idea, to improve transfection success, is thus to perform classical membrane permeabilization allowing plasmid DNA electrotransfer to the cell cytoplasm and, thereafter, when DNA has reached the nuclear envelope, to specifically permeabilize the nucleus using these short strong nanopulses. Thus, when used in conjunction with classical electropermeabilization, nanopulses gave hope to increase gene expression. Another idea is to combine electric pulses and ultrasound assisted with gas microbubbles, known as sonoporation. Cells that received electrosonoporation demonstrated a fourfold increase in transfection level and a sixfold increase in transfection efficiency compared with cells that have undergone electroporation alone (Escoffre et al. 2010). Although electroporation induced the formation of DNA aggregates into the cell membrane, sonoporation induced its direct propulsion into the cytoplasm. Sonoporation can therefore improve the transfer of electro-induced DNA aggregates by allowing its free and rapid entrance into the cells. These results demonstrated that in vitro gene transfer by electrosonoporation could provide a new potent method for gene transfer.

7.3 Imaging Cells and Tissue Processes

7.3.1 3D Cell Culture Models

Historically the culture of mammalian cells for laboratory uses has been performed on Petri dishes. However, it is nowadays clear that 2D studies do not translate well to the 3D microenvironment. Over the last several decades, 2D and 3D tissue engineering approaches have been developed to better mimic the complex architecture and properties of in vivo tissue. Literally, a new dimension to research has been achieved by the advent of three-dimensional cell culture techniques to bridge the gap between the “absolute in vitro” and “true in vivo” (Ravi et al. 2016).

Therefore, in the last past few years, in vitro tissue models, namely, multicellular tumor spheroids and cell sheets, have been used for the understanding of the electrotransfer processes in tumors and skin.

In order to assess the effects of extracellular matrix (ECM) composition and organization as well as intercellular junctions in tissue response to electric pulses, 3D human dermal tissue was reconstructed in vitro by a tissue engineering approach named self-assembly (Madi et al. 2015). This human cell model presented multiple layers of primary human dermal fibroblasts embedded in a native, collagen-rich

ECM. Cells of the reconstructed cutaneous tissue were efficiently electropermeabilized by applying millisecond electric pulses, without affecting their viability. A reporter gene was successfully electrotransferred and gene expression was detected for up to 48 h. Interestingly, the transfected cells were solely located on the upper surface of the tissue, where they were in close contact with plasmid DNA solution. Furthermore, results provided evidence that electrotransfection success depends on plasmid mobility within tissue rich in collagens, but not on cell proliferation status. In addition to proposing a reliable alternative to animal experiments, tissue engineering produces valid biological tool for the in vitro study of gene electrotransfer mechanisms in human tissue. A better comprehension of gene electrotransfer in such a model tissue would help to improve electrogene therapy approaches such as the systemic delivery of therapeutic proteins and DNA vaccination.

In order to in vitro mimic tumors, multicellular spheroids have been developed (Sutherland 1988). Upon growth, spheroids display a gradient of nutrients, metabolites, and proliferating cells. These proliferating cells are located in the outer cell layers, and the quiescent cells are located more centrally. This cell heterogeneity is similar to that found in avascular micro-regions of tumors. Confocal microscopy permits to visualize the repartition of permeabilized cells in spheroids submitted to electric pulses and to unravel gene transfer mechanisms. Experimental results revealed that cells were efficiently permeabilized, whatever their localization in the spheroid, even those in the core. Electrotransfer of bleomycin and cisplatin confirmed the relevance of the model in the case of electrochemotherapy, and doxorubicin showed its potential to screen new antitumor drug candidates for ECT. The combination of antitumor drugs and electric pulses indeed led to changes in spheroid macroscopic morphology and cell cohesion, to tumor spheroid growth arrest, and finally to its complete dislocation, mimicking previously observed in vivo situations (Gibot et al. 2013). In addition, comparison of transfection efficiency between cells in suspension and cells in spheroid allowed highlighting fundamental differences with 2D cell cultures. Using this 3D spheroid cell culture model also allows to study the effect of calcium electroporation and electrochemotherapy using bleomycin on human cancer cell lines and on primary normal human dermal fibroblasts. The results showed a clear reduction in spheroid size in spheroids after treatment with, respectively, calcium electroporation or electrochemotherapy using bleomycin. Strikingly, the size of normal fibroblast spheroids was affected neither after calcium electroporation nor after electrochemotherapy indicating that calcium electroporation, like electrochemotherapy, will have limited adverse effects on the surrounding normal tissue when treating with calcium electroporation (Frandsen et al. 2015).

The spheroid model therefore allows to study and optimize electromediated drug delivery protocols (Gibot and Rols 2013). Small molecules can be efficiently transferred into cells, including the ones present inside the spheroids, but gene expression is limited to the external layers of cells. Taken together, these results are in agreement with the ones obtained by the group of R. Heller in the USA (Marrero and Heller 2012) and indicate that the spheroid model is more relevant to an in vivo situation than cells cultured as monolayers.

7.3.2 Tissue Responses

Besides permeabilization of membranes, another important effect of electroporation that occurs in tissues (and which cannot be observed in vitro) is the blood flow-modifying effect (i.e., vascular lock) (Gehl et al. 2002). Electric pulses indeed induce a blood volume reduction in tumors (Sersa et al. 1999) and a short-term reduction of perfusion in muscles (Gehl et al. 2002). It has been observed that EP induces blood-modifying effects in tumors and normal tissues, but when combined with chemotherapeutics, it also results in disruption of tumor vasculature, without affecting adjacent normal vasculature. This dual effect of electrochemotherapy has clinical importance. On the one hand, due to the vascular disrupting action of ECT, it has proven to be successful in the treatment of bleeding tumors, and on the other hand, it can be safely applied to tumors that lie near large normal blood vessels, as was recently demonstrated in a clinical study on liver metastases of colorectal cancer. Moreover, even when used close to the heart, electroporation proved to be a safe method.

Direct observation with intravital fluorescence digitized microscopy imaging allowed to directly visualize the effects of electric pulses on the subcutaneous blood vessel dynamics and molecule electrotransfer (Bellard et al. 2012). These features were measured in mice via a dorsal skinfold window chamber, using fluorescently labeled dextrans of different sizes. Application of electric pulses on the skin in vivo resulted in a rapid increase in vascular permeability that gradually recovered to basal levels at different times posttreatment, depending on dextran size. Simultaneously, the immediate constriction of the blood vessels occurred which was more pronounced for arterioles compared to venules. This vasoconstriction of arterioles results in a transient "vascular lock." The increased permeability of small vessel walls whatever the dextran size associated with delayed perfusion can explain the improved delivery of the intravenous injected molecules (i.e., drugs, gene delivery) into the tissues induced by electropermeabilization in vivo. The study showed that plasmid DNA is sensitive to vascular lock. Namely, constriction of vasculature delays movement of large-sized molecules through the vasculature wall. Therefore, caution is needed when DNA is administered intravenously.

By using noninvasive bioluminescence technology, it has been recently possible to further explore the phenomena associated with GET to tumors by a real-time monitoring of the transfection efficiency as well as cell death following the treatment. Results showed that the GET of a reporter gene can lead to nonspecific antitumor effectiveness and even complete regression of tumors. Additionally, using the intratumoral GET of a luciferase-encoding plasmid, the source of the expression was localized mainly in the peritumoral and not in the tumoral region. These data provide new insights into some of the phenomena associated with GET into tumors, which should be taken into account when designing improved and more effective cancer gene therapy, in order to accelerate the transfer of the technology into clinical trials (Kamensek et al. 2016).

7.4 Summary and Future Direction

Clinical delivery of cytotoxic drugs is nowadays successfully used to treat tumors in patients. GET was originally used as a laboratory tool to deliver DNA to bacterial and mammalian cells in culture. Pulse generators and electrode developments allowed the method to be successfully used for in vivo uses. Several clinical trials related to delivery of plasmid DNA are promising in both cancer therapeutic and infectious disease vaccine applications. One huge benefit of the electropermeabilization technique besides its safety, efficiency, and low cost is its malleability. Electric pulse protocols can be fine-tuned to control the location, levels, and duration of subsequent transgene expression (Heller and Heller 2015).

Efficient GET is dependent on several cell- and tissue-related factors including extracellular matrix density and composition, plasmid DNA uptake, and nucleocytoplasmic transport. Different barriers are encountered by plasmid DNA from the extracellular environment toward the interior of the cell, and different strategies must be developed to overcome these biological barriers. A better understanding of the cellular and molecular bases of the physical gene transfer process may provide strategies to overcome those obstacles that highly limit the efficiency and use of gene delivery methods (Escoffre et al. 2010). Therefore, it is necessary to develop and use different models, from simple lipid vesicles to tumor multicellular tumor spheroids closer to the in vivo situation, for the understanding of the membrane permeabilization and DNA electrotransfer process in tissues. Each of these models has advantage and limits. When combined they can help to study the complete process (Table 7.1).

We believe that a full comprehension of the process involved in electropermeabilization and gene electrotransfer would further improve therapeutic approaches.

Table 7.1 Models to address electropermeabilization and gene delivery processes

Model	Membrane permeabilization	DNA electrotransfer
GUV	Visualization of membrane effects (deformation, lipid loss)	Failed to mimic DNA/membrane interaction (DNA is directly transferred inside the vesicle)
2D cell culture	Consequences of membrane permeabilization on membrane organization (lateral and transverse mobility of lipids and proteins)	Visualization of DNA/membrane complex formation and DNA traffic into the cells
3D cell culture	Molecules transfer that fairly mimic in vivo complex situations (contacts between cells, junctions, extracellular matrix)	Allow to study DNA delivery in 3D and mimic in vivo situation (decrease in gene expression from the periphery to the core)
Small animal (window chamber)	Visualization of blood flow modifications (vascular lock)	Allow to address in vivo effects (DNA sensitivity to vascular lock and constriction of vasculature delaying movement of large-sized molecules through the vasculature wall)

References

Andre FM, Mir LM (2010) Nucleic acids electrotransfer in vivo: mechanisms and practical aspects. Curr Gene Ther 10(4):267–280

Beebe SJ, White J et al (2003) Diverse effects of nanosecond pulsed electric fields on cells and tissues. DNA Cell Biol 22(12):785–796

Bellard E, Markelc B et al (2012) Intravital microscopy at the single vessel level brings new insights of vascular modification mechanisms induced by electropermeabilization. J Control Release 163(3):396–403

Cemazar M, Jarm T et al (2010) Cancer electrogene therapy with interleukin-12. Curr Gene Ther 10(4):300–311

Chiarella P, Fazio VM et al (2010) Application of electroporation in DNA vaccination protocols. Curr Gene Ther 10(4):281–286

Chopinet L, Roduit C et al (2013) Destabilization induced by electropermeabilization analyzed by atomic force microscopy. Biochim Biophys Acta 1828(9):2223–2229

Daud AI, DeConti RC et al (2008) Phase I trial of interleukin-12 plasmid electroporation in patients with metastatic melanoma. J Clin Oncol 26(36):5896–5903

Escoffre JM, Rols MP (2012) Electrochemotherapy: progress and prospects. Curr Pharm Des 18:3406–3415

Escoffre JM, Teissie J et al (2010a) Gene transfer: how can the biological barriers be overcome? J Membr Biol 236(1):61–74

Escoffre JM, Kaddur K et al (2010b) In vitro gene transfer by electrosonoporation. Ultrasound Med Biol 36(10):1746–1755

Escoffre JM, Portet T et al (2011) Electromediated formation of DNA complexes with cell membranes and its consequences for gene delivery. Biochim Biophys Acta 1808(6):1538–1543

Escoffre JM, Bellard E et al (2014a) Membrane disorder and phospholipid scrambling in electropermeabilized and viable cells. Biochim Biophys Acta 1838(7):1701–1709

Escoffre JM, Hubert M et al (2014b) Evidence for electro-induced membrane defects assessed by lateral mobility measurement of a GPi anchored protein. Eur Biophys J 43:277–286

Faurie C, Rebersek M et al (2010) Electro-mediated gene transfer and expression are controlled by the life-time of DNA/membrane complex formation. J Gene Med 12(1):117–125

Frandsen SK, Gibot L et al (2015) Calcium electroporation: evidence for differential effects in normal and malignant cell lines, evaluated in a 3D spheroid model. PLoS One 10(12):e0144028

Gehl J, Skovsgaard T et al (2002) Vascular reactions to in vivo electroporation: characterization and consequences for drug and gene delivery. Biochim Biophys Acta 1569(1–3):51–58

Gibot L, Rols MP (2013) Progress and prospects: the use of 3D spheroid model as a relevant way to study and optimize DNA electrotransfer. Curr Gene Ther 13(3):175–181

Gibot L, Wasungu L et al (2013) Antitumor drug delivery in multicellular spheroids by electropermeabilization. J Control Release 167(2):138–147

Golzio M, Teissie J (2014) siRNA delivery via electropulsation: a review of the basic processes. Methods Mol Biol 1121:81–98

Golzio M, Teissie J et al (2002) Direct visualization at the single-cell level of electrically mediated gene delivery. Proc Natl Acad Sci U S A 99(3):1292–1297

Heller LC, Heller R (2010) Electroporation gene therapy preclinical and clinical trials for melanoma. Curr Gene Ther 10(4):312–317

Heller R, Heller LC (2015) Gene electrotransfer clinical trials. Adv Genet 89:235–262

Kamensek U, Rols MP et al (2016) Visualization of nonspecific antitumor effectiveness and vascular effects of gene electro-transfer to tumors. Curr Gene Ther 16(2):90–97

Madi M, Rols MP et al (2015) Efficient in vitro electropermeabilization of reconstructed human dermal tissue. J Membr Biol 248:903–908

Marrero B, Heller R (2012) The use of an in vitro 3D melanoma model to predict in vivo plasmid transfection using electroporation. Biomaterials 33(10):3036–3046

Mauroy C, Castagnos P et al (2012a) Interaction between GUVs and catanionic nanocontainers: new insight into spontaneous membrane fusion. Chem Commun (Camb) 48(53):6648–6650
Mauroy C, Portet T et al (2012b) Giant lipid vesicles under electric field pulses assessed by non invasive imaging. Bioelectrochemistry 87:253–259
Mir LM, Glass LF et al (1998) Effective treatment of cutaneous and subcutaneous malignant tumours by electrochemotherapy. Br J Cancer 77(12):2336–2342
Neumann E, Schaefer-Ridder M et al (1982) Gene transfer into mouse lyoma cells by electroporation in high electric fields. EMBO J 1(7):841–845
Paganin-Gioanni A, Bellard E et al (2011) Direct visualization at the single-cell level of siRNA electrotransfer into cancer cells. Proc Natl Acad Sci U S A 108(26):10443–10447
Portet T, Camps i Febrer F et al (2009) Visualization of membrane loss during the shrinkage of giant vesicles under electropulsation. Biophys J 96(10):4109–4121
Portet T, Favard C et al (2011) Insights into the mechanisms of electromediated gene delivery and application to the loading of giant vesicles with negatively charged macromolecules. Soft Matter 7(8):3872–3881
Ravi M, Ramesh A et al (2016) Contributions of 3D cell cultures for cancer research. J Cell Physiol 232(10): 2679–2697
Rols MP, Delteil C et al (1998) In vivo electrically mediated protein and gene transfer in murine melanoma. Nat Biotechnol 16(2):168–171
Rosazza C, Escoffre JM et al (2011) The actin cytoskeleton has an active role in the electrotransfer of plasmid DNA in mammalian cells. Mol Ther 19(5):913–921
Rosazza C, Buntz A et al (2013) Intracellular tracking of single plasmid DNA-particles after delivery by electroporation. Mol Ther 21:2217–2226
Rosazza C, Meglic SH et al (2016) Gene electrotransfer: a mechanistic perspective. Curr Gene Ther 16(2):98–129
Sersa G, Cemazar M et al (1999) Tumor blood flow modifying effect of electrochemotherapy with bleomycin. Anticancer Res 19(5B):4017–4022
Sersa G, Teissie J et al (2015) Electrochemotherapy of tumors as in situ vaccination boosted by immunogene electrotransfer. Cancer Immunol Immun 64:1315–1327
Sutherland RM (1988) Cell and environment interactions in tumor microregions: the multicell spheroid model. Science 240(4849):177–184
Teissie J, Golzio M et al (2005) Mechanisms of cell membrane electropermeabilization: a minireview of our present (lack of ?) knowledge. Biochim Biophys Acta 1724(3):270–280
Yarmush ML, Golberg A et al (2014) Electroporation-based technologies for medicine: principles, applications, and challenges. Annu Rev Biomed Eng 16:295–320