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Dosimetry in Electroporation-Based Technologies and Treatments

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To measure is to know.
If you cannot measure it, you cannot improve it.

Lord Kelvin

12.1 Introduction

Electroporation is a platform technology that is already established in medicine and food processing (Haberl et al., 2013a). It is based on increased cell membrane permeability due to exposure to electric pulses (Weaver, 1993; Kotnik et al., 2012). If the cell is able to fully recover afterwards, we call it reversible electroporation; when the damage is too great and the cell dies, we call it irreversible electroporation (IRE). Electrochemotherapy (ECT) is an antitumor therapy in which locally applied high-voltage (HV) pulses trigger a transient permeabilization of tumor cells. Diffusion of a chemotherapeutic drug (bleomycin or cisplatin) is enabled, resulting in higher cytotoxicity. Effectiveness is accomplished if sufficient drug concentration and electric field in the tumor are achieved. ECT is a highly efficient treatment, with complete response rates of between 60% and 70% on a single treatment and with objective response rates up to 80% (Mali et al., 2013),

and is comparable to, if not more efficient than, other similar skin-directed technologies (Spratt et al., 2014). It is used in the treatment of cutaneous and subcutaneous tumors, following standard operating procedures (SOPs; Mir et al., 2006).

If the exposure of the cell to electric field is too excessive, it dies, presumably due to a loss of homeostasis. IRE is used as an ablation method for normal and tumor tissues. It is called "nonthermal" ablation, because cells primarily die due to membrane permeabilization and not due to the increase in the temperature of the tissues. However, we should not overlook local temperature increases around the electrodes, which can be significant at higher amplitudes, increased duration, or number of pulses (Garcia et al., 2014; Kos et al., 2015). Considerable research has also been undertaken in the area of gene transfection and biopharmaceutical drugs stimulating an immune response. Gene electrotransfer (GET) is a nonviral method for delivering DNA molecules into cells. DNA vaccination using electric pulses and clinical trials of GET of DNA with interleukin-12 in patients with metastatic melanoma also has shown great promise in clinical practice (Heller et al., 2001; Haberl et al., 2013a; Lambricht et al., 2016).

Many of its biotechnological applications such as inactivation of microorganisms and extraction of biomolecules have only recently started to emerge, while nonthermal food pasteurization is already being used in the industry (Toepfl et al., 2006; Kotnik et al., 2015). Electroporation is more commonly termed as pulsed electric field (PEF) treatment in food technology. Food preserved by PEF maintains color and flavor, and the antioxidant levels also stay unaffected (Haberl et al., 2013). It is efficient for increasing the shelf life of liquid food (Toepfl et al., 2006). A combination of mild preheating to 60°C and subsequent electroporation reduces the energy needed for efficient disinfection to 40 kJ/L (Gusbeth and Frey, 2009).

Microalgae are currently the most intensely investigated feedstock for biomass production with electroporation; they are getting implemented in biofuel applications (Golberg et al., 2016; Postma et al., 2016). A combination of grape fermentation and electroporation led to an increased content of polyphenolic compounds and less acidity, thereby resulting in a slightly smoother taste and color intensity in wine (Mahnič-Kalamiza et al., 2014). Overall, it is a fast-growing field with great potential.

12.1.1 Electroporation—The Phenomenon

Electroporation is a phenomenon in which cells that are exposed to a high enough electric field increase permeability and conductivity of their membranes. Each biological cell is surrounded by a membrane that mainly consists of phospholipids. Lipids in aqueous conditions spontaneously form a two-molecule thick layer as a result of their dielectric properties. Water and water-soluble molecules cannot pass the entirely intact barrier only by diffusion (Deamer and Bramhall, 1986). In addition, biological membrane also contains glycolipids, cholesterol, and various proteins, which enable selective transport of some molecules from intracellular space to the cell interior and vice versa (Kotnik et al., 2012).

Several theoretical descriptions of the electroporation phenomenon have been proposed. The most established and likely one is that electroporation is based on the aqueous pores formation in the lipid bilayer (Freeman et al., 1994; Weaver and Chizmadzhev, 1996; Kotnik et al., 2012). When cells are exposed to a high pulsed electric field, voltage

is induced across their membranes. This results in the rearrangement of their membrane components, leading to the formation of hydrophilic pores in the bilayer, the presence of which increases the ionic and molecular transport to otherwise impermeable membranes (Pucihar et al., 2008; Kotnik et al., 2012). Experimental observation of the pore formation was not successful with known techniques, but molecular dynamic (MD) simulation provides convincing corroboration. From the electrical point of view, a cell can be modelled as an electrolyte (conductive media), surrounded by an electrically insulated/dielectric shell. Each cell under physiological conditions has a resting transmembrane voltage in the range of −90 to −40 mV (Kotnik et al., 2010). This is a result of ion imbalance in the cytoplasm, controlled by Na^+–K^+ pumps and K^+ leak channels. Na^+–K^+ pumps export Na^+ ions out of the cell and simultaneously import K^+ ions; meanwhile, K^+ ions can freely cross the membrane through K^+ leak channels, to achieve electrical and concentration equilibrium. Applied electric pulses cause local field distortion in the cell and their surroundings. Due to low-membrane conductivity in the vicinity of the cell, the electrical field concentrates mainly in the cell membrane, resulting in electrical potential difference across the membrane. The induced transmembrane voltage superimposes to the resting potential. It can affect transport through the membrane, stimulate cells, and if high enough, lead to the electroporation of cell membrane. Increased cell permeabilization is observed with electric field increase; induced transmembrane voltage is dependent on position, cell shape, and orientation. Delay between external and inducted voltage is in the microsecond range and is determined by the membrane time constant τ_m (Isokawa, 1997). If cells are exposed to electric field in low conductivity medium, delay significantly increases (Kotnik et al., 2010). When short, intense electric pulses (nanosecond pulses, tens of kV/cm, with a period similar to τ_m or shorter) are applied, the outer membrane acts as a short circuit because of cell frequency response, and the applied voltage also appears across the interior of the cell (Kotnik and Miklavčič, 2006). Nanosecond pulses can induce a high enough voltage to cause electroporation of internal organelles (Batista Napotnik et al., 2016). Because organelle interior is electrically more conductive then cytosol, and organelle membrane dielectric permittivity is lower than a cell membrane permittivity, a voltage induced on organelle membrane can exceed the one induced on the cell membrane, resulting in increase of induced voltage amplitude (Kotnik et al., 2010; Retelj et al., 2013). But at the same time, pulses also cause plasma membrane permeabilization (Kotnik et al., 2006; Batista Napotnik et al., 2010).

12.1.2 Physical Dosimetry in Electroporation

The local electric field, i.e., the one "felt" by the cell is the one that leads to membrane electroporation. Applicator/electrode characteristic and applied pulse characteristics define the electric field distribution and intensity. For various applications, different pulse shapes, voltages, duration, repetition frequencies, and sequences are needed. Therefore, special pulse generators have been designed called electroporators. Because biological load characteristics vary considerably, and in addition their conductivity changes due to electroporation during the pulse delivery, development of these devices is challenging.

12.1.2.1 Dosimetry of Pulse Delivery

The electroporation signal is, as mentioned before, characterized by pulse amplitude, shape and duration, number of pulses, pulse repetition frequency, and pulse orientation sequence. Most common pulse shapes that are used in electroporation are square wave (also bipolar), exponential decay, and bell-shaped pulses. When designing an electroporation device, one should always keep in mind that a biological sample as a load has a resistive–capacitive nature and can vary from sample to sample, and in addition the impedance of a biological sample decreases during pulse delivery (Pliquett et al., 2000; Pavlin et al., 2005). The most simple and inexpensive way to generate pulses is by a capacitor discharge circuit (Figure 12.1). When we are dealing with higher voltages, it is more efficient and easier to use smaller capacitors and connect them in to a Marx generator (Figure 12.1c). The main problem here becomes simultaneous switching; the switching element must be chosen with respect to their maximum operating voltage and response rate. The generated pulses have typical capacitor discharge-exponentially decay shape (Reberšek and Miklavčič, 2011; Reberšek et al., 2014). Micro- and millisecond square pulses are usually generated by an HV power supply switching circuit (Figure 12.2a), with fast-power MOSFET (Metal-Oxide-Semiconductor Field-Effect Transistor) or IGBT (Isolated-Gate Bipolar Transistor) used as the switch. All the required energy must be generated and stored in the capacitor before delivery. To minimize a voltage drop, a very large capacitor is needed, resulting in difficult voltage modification. We also have

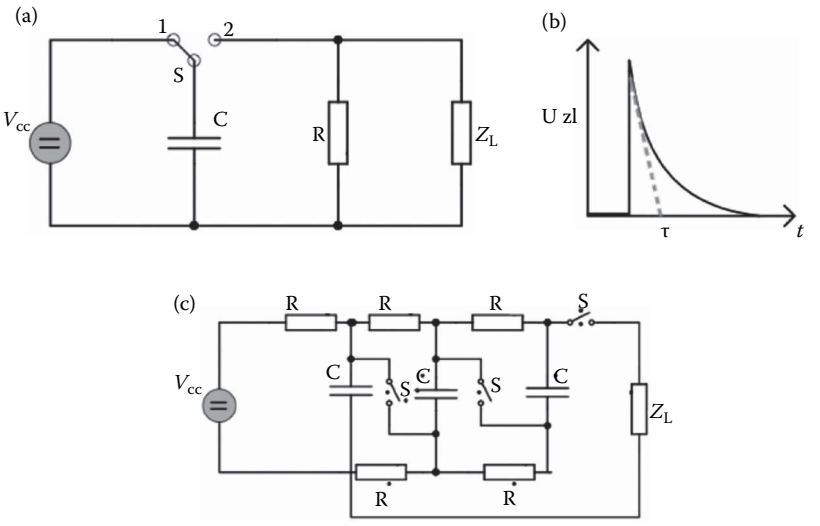


FIGURE 12.1 Panel (a) is capacitor discharge circuit, a built-in resistance R is added to limit the decrease of time constant $\tau = C * |Z_L| \cdot I_F * |Z_L| \geq 10R, \tau \approx RC$, resulting in 90% energy dissipation through R. Panel (c) is a proposed Marx bank circuit. Capacitors C are charged in parallel through resistor R and then switched to series building up the voltage to $n * U$ and discharged through the load Z_L, by switching all switches simultaneously. The maximum applied voltage is equal to the load power supply voltage multiplied by the number of capacitors and time constant. In panel (b), a generated pulse shape is presented, the discharge time is time constant dependent.

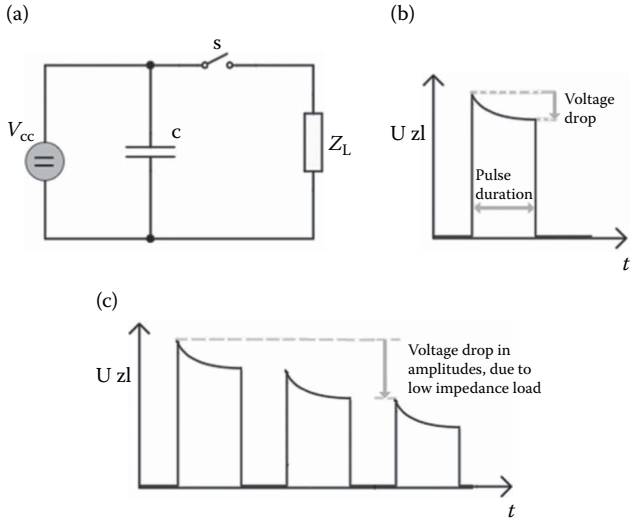


FIGURE 12.2 High-voltage power supply switching circuit (a). The variable power supply V_{CC} defines the amplitude of the output pulse. Switches control pulse duration and pulse repetition frequency. The voltage drop that occurs during pulse is proportional to load impedance $\left(\text{voltage drop} = \text{pulse duration}/\left(C^*\left|Z_L\right|\right)\right)$ (b). In (c) C an example of reduced amplitude is shown, that can occur in a case of low impedance load that requires too high current.

some time limitations; pulses can be generated after the capacitor is recharged to the preset voltage. Low impedance of a load (tissue or cell suspension) requires large power/currents, which quite often leads to a significant voltage drop. Protocols in which a larger number of pulses are delivered can result in reduced amplitude of pulses (Figure 12.2). This is one of the main reasons why we need to measure when using devices for electroporation. Furthermore, we focus on measuring and quality control.

To achieve an efficient electric field that enables electroporation, we are dealing with HV and currents; therefore, generator construction can be challenging. The shorter the pulses, the more complicated the circuit designs that are required; it is really challenging to generate high-power and short-duration pulses. HV switches with short rise times are needed; spark gaps, UV lasers, SiC MOSFETs, or IGBTs can be used, depending on the application. With nano- and picosecond pulses, pulse-forming networks are a common solution, e.g., a transmission line (Figure 12.3a). Transmission lines operate in both charging and discharging phases. Generated energy should be stored in a large capacitor and then discharged to the load. After pulse generation, a new pulse can be delivered when the capacitor is recharged, resulting in repetition frequency limitation (Bertacchini et al., 2007; Syamsiana and Putri, 2011; Reberšek et al., 2014).

Electrodes, together with the biological sample, present the load for the pulse generator. The main problem that we encounter here is that electrodes get polarized where they get in touch with the sample due to water molecules and hydrated ions that are present in the surrounding area. It is a frequency-dependent phenomenon, which can be modeled as a capacitor in series with a resistor (Chafai et al., 2015). In a cell suspension, a counterion

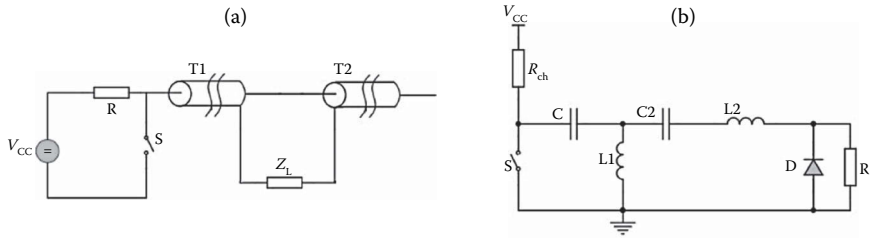


FIGURE 12.3 Concept of Blumlein transmission line (a) and diode opening switch (DOS) circuit (b), commonly used in nanosecond pulse generator designs. The Blumlein transmission line generators have a variable high-voltage power supply *V*, a charging resistor *R*, and two transmission lines. T1 and T2 are charged when the switch is turned off and then discharged through the load when switched. The pulse duration cannot be modified as it equals twice the electrical length of the transmission line, if the impedance of the load is twice the impedance of the transmission line. The DOS generators can be composed of more accessible electrical components than Blumlein generators. Pulse is formed by a diode that must be forward and reverse pumped with adequate sinusoidal current. Diode should stop conducting when the majority of the total energy is stored in L2. That means a current must be maximized at the time of switching. High voltage is switched by diodes, which means MOSFET-s does not need to withstand the whole output amplitude, and does not need to be faster than the output pulse. Pulse duration is determent with diode reverse recovery time. But finding the appropriate matching of capacitors and inductors values in LC oscillator for optimal switching can be challenging. (From Reberšek, M., et al., *IEEE Electr. Insul. Mag.,* 30(3), 8–18, 2014; Sanders, J. M., et al., *IEEE Trans. Dielectr. Electr. Insul.,* 16(4), 1048–1054, 2009.)

layer is formed at each electrode and electric field driving charge transport is reduced, resulting in lower suspension conductivity. At the contacts with tissue, electrodes stimulate the release of electrolytes, resulting in the development of a poorly conductive region where wounds can occur. Luckily, polarization decreases with increasing frequency.

Electrodes must be user friendly; the wires connecting them should be long enough to enable easy handling and smooth application. But each additional wire/connection has some parasitic properties resulting in route losses—the higher the frequencies, the more the parasitic characteristics are manifested. In worst cases, when dealing with nanosecond pulses, the generated pulses at the end of electrodes can completely differ from the ones at the output stage of the electroporator. At high frequencies, reflection on the lines must also be taken into account. The thickness of the wire must be compatible with the output current (Kolb et al., 2006; Batista Napotnik et al., 2016).

Different electrodes are available on the market, and they need to be chosen considering the targeted load and pulse generator restrictions. Electrode geometry and position also determine electric field distribution. *In vitro*, four main groups of electrodes are present: single-cell chambers, macroelectrodes (two-plate electrodes separated for at least 1 mm), microelectrodes (glued onto cover glass, with separation of 100 μm), and flow-through chambers (polyethylene or polypropylene used as insulating materials, combined with stainless steel electrodes) (Reberšek et al., 2014). When using nano- or picosecond pulses, impedance matching must be ensured. For *in vivo* use macroplate and needle electrodes are commonly used. Electrochemotherapy standard operating

procedure (ESOP) (Mir et al., 2006) describes three different types of electrodes that were developed within the Cliniporator project and are compatible with the Cliniporator generator (Table 12.1). According to SOP, plate electrodes are recommended for treatment of small and superficial tumor nodules. For treatment of thicker and deeper-seated tumor nodes, needle electrodes are more suitable. During the development of Cliniporator, voltage characteristics—sequences—were determined and acquired for better efficacy (Ongaro et al., 2016). Sometimes, large volumes need to be treated; lately, a new type of electrode that covers larger surfaces has become a subject of study (Ongaro et al., 2016).

12.1.2.2 Measuring

When dealing with electroporation, measuring is crucial for achieving effective electroporation. Quality assurance can only be provided by appropriate measurements. Unfortunately, failed efforts to confirm other group's published work are increasing (Kaiser, 2016). The description of the used equipment and the process are flawed. In many papers describing/using electroporation, insufficient detail is reported, and quite often measurements are not reported (Batista Napotnik et al., 2016; Campana et al., 2016). The electroporation field needs to promote research reproducibility, and to improve this, we further need to try and answer the following questions: "Why measuring is necessary?", "How to measure?", "Which data are significant for researches?", and "Which electroporator to choose?"

For electroporation, you need an electroporator. A considerable number of electroporation devices can be found in the market, some are designed for specific applications and some are multifunctional. Most often, they are compatible with different electrodes. It seems however that as the market grows, manufacturers tell us less about their devices. We have already reached a critical point in the field of gene transfection, where preprogramed electroporation procedures are most commonly used so that quite often the researcher does not even know basic pulse parameters, such as pulse shape, repetition rate, and even less about applied voltage amplitude/electric field strength (see Tables 12.1 and 12.2). The researchers are only aware of the program number they used on their device. This limited information availability restricts and hampers the development of new knowledge. In this case, preengineering of electroporation devices limits researchers and hampers the sharing and comparing of results or protocols and even the further development of gene transfection field.

The most complicated of all are nanosecond electroporation systems. They usually consist of nanosecond pulse generator, transmission line or delivery system, and electroporation chamber/electrodes (Pakhomov et al., 2009; Ibey et al., 2010; Batista Napotnik et al., 2016). When using nanosecond pulses, it should always be taken into account that pulses reflect on impedance change and lose power in the transmission line. If the impedance matching is not guaranteed, reflections are present and load dependent. When load impedance is higher, reflections are positive and add to amplitude that would be present on a matched load. In case of lower load impedance, amplitude on the load will be lower. Pulses can become bipolar and cancelation effect can occur. Nanosecond pulses travel approximately 20 cm/ns in coaxial cable (Batista Napotnik et al., 2016).

12.1.2.3 Why Is Measuring Necessary?

For quality assurance! To be sure that the pulses are delivered and the device operates according to its specifications.

The first and most logical answer is because we want to know if our pulses were successfully delivered and we need to know what was delivered. If the current flows through a load, a delivery was more or less successful. But we still do not know anything about the pulse shape and voltage amplitude, or how many pulses were actually delivered. Due to the nature of electric discharge circuits that are commonly used in electroporation devices, amplitude of successive pulses can be lower with each successive pulse delivered (Figure 12.4), if the pulse repetition rate is in the higher half of device operation range. Low-conductivity media in cuvettes is used for two reasons—to reduce heating of the sample due to the current flow and to facilitate pulse generation. With lower resistant loads, problems can occur because the pulse generators cannot deliver "what they promise," i.e., high currents. A typical cuvette resistance with a low conductivity media is somewhere between 100 and 50 Ω. But high conductive media can have a resistance between 10 and 15 Ω, and even lower, requiring even up to 10 × higher currents. This large variation of load characteristics represents a great challenge in electroporator design resulting in different solutions. Because of software or hardware errors, devices can have an unexpected delay during generation, one or more pulses may be omitted, a voltage amplitude may deviate from the expected value, etc. In the case of clinical medical devices with CE certification, these errors should not occur, or if they do, an alarm must be triggered. But when we are dealing with self-developed or commercial devices not classified as medical devices, monitoring is necessary. Also, electronic components and, consequently, devices are aging, and their characteristics may change with time. Built-in measurement systems are usually comfortable solutions, but some manufacturers are taking shortcuts. If the measurement system is a part of the device, it needs periodic calibration; so if this is not part of the unit maintenance, the measurement system is questionable.

The second reason why measuring is necessary is the reproducibility of research results. For research reproducibility, at least similar, if not exactly the same, pulses are needed. Different research groups have different electroporation devices whose output pulses may derogate from specified shapes/parameters. If we know exactly what kind of pulse is needed, a custom setting might lead us towards better matching. Not reporting pulse parameters hinders the comparison of results and hinders progress of research.

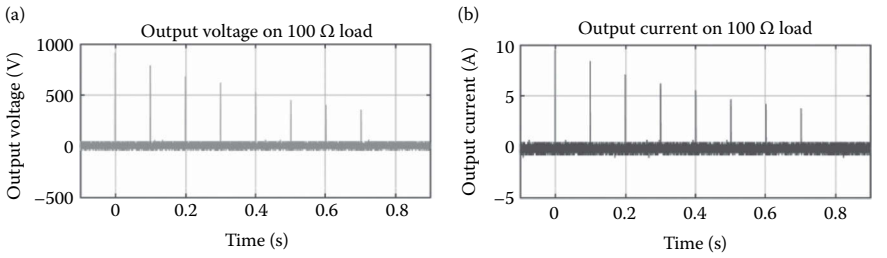


FIGURE 12.4 An example of output pulse measurements, we used a SENNEX electroporator with surface pin electrodes. The biological load was simulated with a 100 Ω resistor. Amplitude of each successive pulse is lower, due to improper operation. The last pulse does not even reach 50% of the preset amplitude. Panel (a) shows output voltage measured on 100 Ω load and (b) output current.

12.1.2.4 How and What to Measure and How to Report

Only correct measurement will upgrade our research, reduce resources used, and help advance the field by contributing to enhancing/improving reproducibility. The easiest way is to measure applied voltage and current. We need an adequate measurement from our oscilloscope and probes. What is appropriate depends on what we want to measure. First, we need to know what pulses are expected—at least amplitude, repetition frequency, and pulse duration. All oscilloscopes have limitations; a bandwidth tells us how fast it follows the signal changes, or more theoretically said, the maximum frequency range that it can measure (Figure 12.8). Closely related to frequency bandwidth is rise time specification. The specified rise time of an oscilloscope defines the fastest rising pulse it can measure. If not specified, it can be calculated as Rise time = 0.35/Bandwidth. For most applications, micro- or millisecond pulses are used; oscilloscopes and probes with a few MHz bandwidth are thus suitable. Measurement gets complicated when we reach the nanosecond HV pulses, where GHz range bandwidths and high rise times are needed. To minimize stray inductance and capacitances and reflections on lines, probes should be located as close to the electrodes as possible, with no additional connecting wires.

For the adequate presentation of pulses delivered, we propose to attach at least two measurements to your publications. One of a single pulse zoomed and another with reduced time scale, where all delivered pulses are displayed (if the number of pulses is low enough to keep a measurement meaningful) (Figure 12.5). If attaching the measurement does not appear to be suitable, an adequate description of a pulse, common notations, and pulse parameters are required. But in some cases when pulses strongly deviate from classical forms, an image tells us a lot more. An example of pulse characteristics determination is given in Figure 12.7.

Exponential decay pulses (Figure 12.6a) are best described by their maximum value, A_{MAX}, and time constant, τ. The value of time constant depends on circuit output stage characteristics. It is defined as the time maximum amplitude A_{MAX}, which drops to 37% of A_{MAX}. Square wave pulses are described with amplitude at high stage (that is choose to best fit the high level) and time t_{FWHM} (Full Width at Half Maximum-FWHM). t_{FWHM} is best described as the time passed between when the pulse reaches 50% of maximal amplitude at the rising and falling phase. Other pulse shapes are best described if we

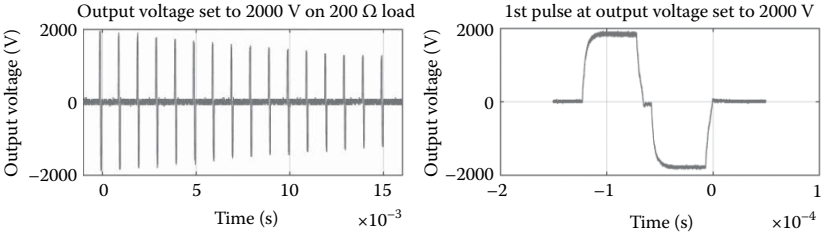


FIGURE 12.5 An example of proposed measurements to accompany the report. For the adequate presentation of pulses delivered, we propose that you attach at least two measurements to your publications. One of a single pulse zoomed and another with reduced time scale, where all delivered pulses are displayed (if the number of pulses is low enough to keep a measurement meaningful) The example measurements were made with the help of a CHEMIPULSE IV electroporator.

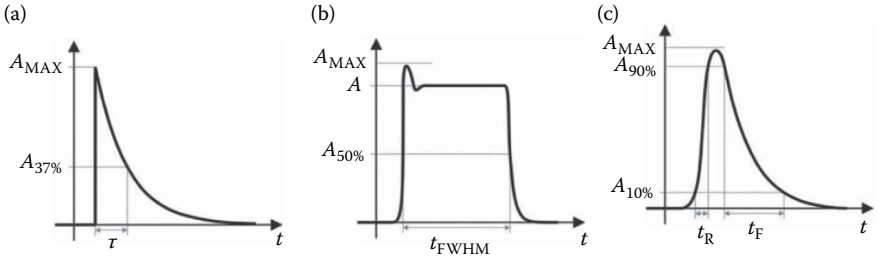


FIGURE 12.6 (a) Exponential decay pulse, (b) square wave pulse, and (c) Gaussian or bell-shaped pulse.

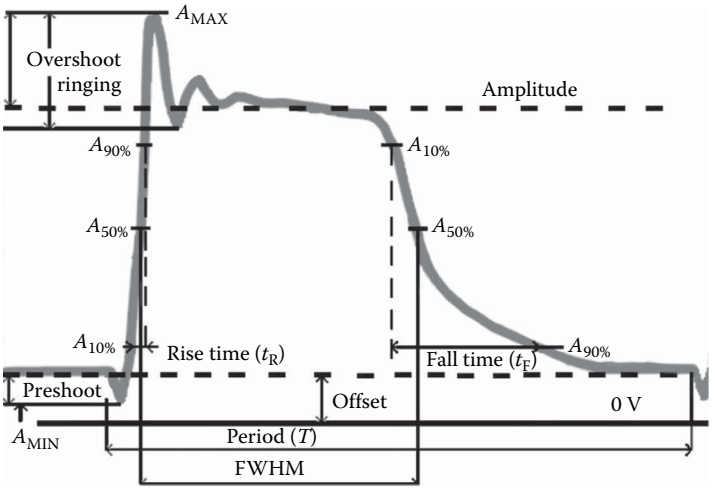


FIGURE 12.7 Pulse characteristics: a presentation of useful terms for description.

define their rise (t_R) and fall times (t_F) and maximum amplitude A_{MAX}. Rise time is time required for a pulse to rise from 10% to 90% of its steady value. Similarly, fall time is the time taken for the amplitude of a pulse to decrease from a specified value (usually 90% of the peak value exclusive of overshoot or undershoot) to another specified value (usually 10% of the maximum value exclusive of overshoot or undershoot) (Reberšek et al., 2014).

12.1.3 Biophysical Dosimetry in Electroporation

Biological cells can be electroporated in suspension, attached, or in tissue. We distinguish *in vitro* and *in vivo* electroporation; the connection between them is not taken for granted. New applications of medical electroporation are first demonstrated *in vitro*, if their efficacy is shown also *in vivo* in an appropriate animal model, human clinical studies can be done (Hofmann., 2000). Electric pulse parameters for effective *in vivo* application can be determined from *in vitro* experiments considering application specifications (Maček-Lebar et al., 2002).

12.1.3.1 Electroporation *In Vitro*

Cell membrane electroporation and consequently increased membrane permeability is controlled by the electric field strength. Because cells are in suspension and we usually work with low cell volume fractions, we can assume the surrounding field is homogenous and uniform throughout all the conducting media (Susil et al., 1998; Kotnik et al., 2010). But induced transmembrane voltage is not uniform on the cell surface, it is dependent on cell size, membrane characteristics and orientation to the field, frequency, and time and space (Teissié and Rols, 1993). *In vitro,* we are dealing with dilute cell suspensions, where the local field outside cells does not affect other cells. If volume fractions are higher than 10%, the induced transmembrane voltage cannot be easily estimated by Schwan's equation; local cell fields influence each other, and therefore approximate analytical or numerical calculations are needed (Pavlin et al., 2005). Even if we increase cell volume fraction of cells, there is still a big difference between tissue and a dense suspension. Plated cells are permeabilized with lower electroporation parameters than when in suspension (Towhidi et al., 2008). In tissues, cells form specific structures and are in contact with each other (Kotnik et al., 2010). *In vitro* experiments can be performed in electroporation chambers, especially with short pulse durations (nano- and picosecond pulses); chamber characteristics such as frequency responses can have a great impact on the results. Results are different when using different cuvettes; pulses are usually applied through two electrodes; the field delivered in is consequently different. From *in vitro* to *in vivo*, one needs to keep in mind that electric pulses are much larger compared to diameters of the cells (Maček-Lebar et al., 2002).

12.1.3.2 Electroporation *In Vivo*

In case of *ex vivo* electroporation of tissues, or *in vivo* electroporation, the electric field can no longer be considered homogenous because tissue is a highly inhomogeneous conductor. Some biological materials are also anisotropic, and therefore electric field orientation must also be considered. Tissue inhomogeneity is frequency dependent, it varies from tissue to tissue and is smaller at higher frequencies. Tumors mostly have a higher water content as a result of cellular necrosis (Miklavčič et al., 2006). In preclinical and clinical studies a few years ago, authors often considered the treated tissues as being linear electric conductors (i.e., with constant tissue conductivities) (Čorović et al., 2013).

Cell membranes have low electric conductivity in comparison to cytoplasm and extracellular medium. Electroporation changes the conductivity of cells, and thereby the field distribution is changed (Sel et al., 2005). To analyze tissue electroporation, we need to know the characteristics of the treated tissue. A macroscopic description is the most common and is described by specific conductivity and relative permittivity. Applied voltage rests among the most resistive tissue, which in the case of external electroporation is the skin. Skin conductivity is 10–100 times lower than the tissue underneath. Restive heating occurs and should be considered so as to avoid damaging healthy cells (Lacković et al., 2009; Kos et al., 2012). Numerical methods are used to define the local electric field distribution within the tumors (Miklavčič et al., 2010; Edhemovic et al., 2011). For deep-seated tumors and tumors in internal organs, which are surrounded by tissues with different electric properties, individualized

patient-specific treatment planning is required (Miklavčič and Davalos, 2015). Tumors vary in shape, size, and location. The shape and position of the used long-needle electrodes and even the applied voltage (Hjouj and Rubinsky, 2010; Edhemovic et al., 2011; Pavliha et al., 2012) are analyzed and optimized for each tumor; coverage of the whole tumor with a sufficiently high electric field (which is one of the two prerequisites for successful treatment) (Miklavčič et al., 1998, 2006) can currently only be assured by means of numerical modeling of electric field distribution (Pavliha and Kos, 2013). Electric field calculations based on real input data are performed. Image-guided

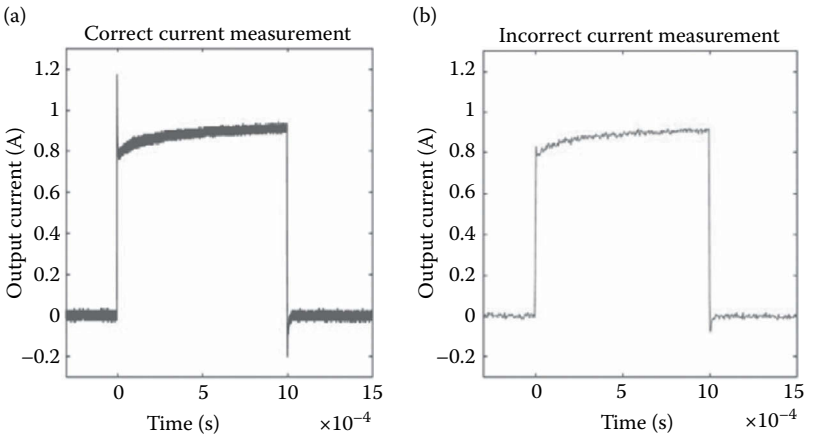


FIGURE 12.8 Current measurement examples, in (b) an oscilloscope with a too low bandwidth was used, consequently current spike was not detected. The spike is clearly visible in (a), that was captured by a faster oscilloscope.

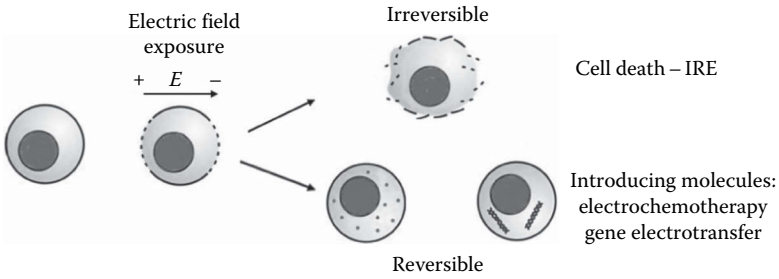


FIGURE 12.9 Symbolic representation of different electroporation applications. When externally applied electric field reaches the cell membrane threshold value, the cell gets permeabilized. We distinguish reversible and irreversible electroporation. The result of irreversible electroporation is cell death, which we exploit for nonthermal ablation, so-called IRE. In case of reversible electroporation, cell membrane can fully recover after the electroporation process. During the electroporation process, molecules are introduced into the cell at electrochemotherapy and gene electrotransfer.

insertion of electrodes is used (Kos et al., 2010; Miklavčič et al., 2010; Grošelj et al., 2015). Nonthermal IRE is also an electroporation-based application that is used for ablation of pathological tissue, it similarly requires a specific treatment planning. But in that case, calculations of temperature increase should also be considered (Županič and Miklavčič, 2011) as the conductivity increases with temperature. It is also necessary to measure *in vivo,* the voltage and current measurement of applied pulses and electrical impedance tomography (EIT) are common, but they do not tell us, how the electrical conductivity of tissue affects the electric field distribution. MR-EIT (Magnetic Resonance-Electrical Impedance Tomography) enables reconstruction of electric field distribution by measuring the electric current density distribution and electric conductivity during electroporation by using MR imaging and numeric algorithms (Kranjc et al., 2015).

12.2 Applications

Further on We will focus on three main applications (Figure 12.9). The most established ECT, IRE, is used for tissue ablation and gene electrotransfection. In each section, we review pulses used, their characteristics, and main principles. For each application, we tried to discover if researches report adequate data. At the end of the chapter, a review of commercially available electroporation devices can be found, including their characteristics. We have summarized all the important parameters, so as to help researchers select the appropriate device for their application. Table 12.2 describes their specifications and limitations. We focused on devices available for ECT, IRE, and gene electrotransfer. After a detailed review of the manufacturer's Internet pages and literature, we wrote to all the listed producers and asked them to kindly review the data we found in literature, device specifications, and on the Internet and update if necessary. We wrote to 25 producers: 13 were pleased to cooperate, the data they provided can be found in Tables 12.1 and 12.2. Three producers (LONZA, MaxCyte, and Ichor Medical Systems) replied to our email and informed us that the requested information was confidential. The data collection lasted for 1 month, with one reminder email. We contacted producers through their official emails published on their homepages. In addition, we also wrote directly to employees, whose contact information we have in our database. There have been no responses from BIO-RAD, Eppendorf, NEPA GENE, Oncosec, Scientz, Sigma-Aldrich, Thermo Fischer, and Tritech Research. Overall, the ones that do not specify their pulse parameters did not change their mind, only Inovio is excluded. We could not find any technical specifications or generated pulse descriptions of their devices, but in the end they provided all the missing data. Most of the producers who did not cooperate sell devices that are mainly used in the biopharmaceutical drug industry. When we are working with a device that has a CE mark, a small derogation from the specification is allowed. But due to aging and the huge diversity in biological load characteristics, control with an external measuring system is required for quality assurance. Noncertified devices can generate pulses that highly deviate from the preset values, so the use of an external measuring system is necessary.

12.2.1 Electrochemotherapy

One of the leading applications on the electroporation field is ECT. It is highly effective, with complete response rates between 60% and 70% and objective response rates of about 80% (Mali et al., 2013). It is suitable for treatment of cutaneous and subcutaneous tumors of different histotypes, both skin and nonskin cancers, as well as metastases. European Standard Operating Procedures of Electrochemotherapy (ESOPE) have been established in 2006; it increased reproducibility and improved clinical practice results (Campana et al., 2016). SOP however only defines ECT for smaller skin tumors (<3 cm in diameter); we still do not have any guidelines for internal or larger tumor ECT. National Institute for Health and Care Excellence (NICE) has recognized ECT as an integral part of the multidisciplinary treatment for patients with skin metastases of nonskin origin and melanoma (NICE interventional procedure guidance IPG 446, http://www.nice.org.uk/guidance/ipg446) (Campana et al., 2016). Lately, ECT has been introduced into the treatment of deep-seated tumors, it is really suitable for treatment of liver metastases, especially when they are located close to major blood vessels and consequently not manageable with surgery (Edhemovic et al., 2014). Recently, recommendations for improving the quality of reporting clinical ECT studies has been released (Campana et al., 2016), on initiative of the Steering Committee of the COST TD 1104 Action. Really good guidelines could raise the level of research even higher. That is a good example for other, high-quality applications. Standardized reporting enables faster and greater progress (Miklavčič et al., 2014).

But the main challenge is still the successful use of the application, the presence of a cytotoxic agent within tumor tissue, and adequate coverage of the tumor with electric pulses above the threshold of reversible membrane electroporation are crucial. Some studies have been conducted that have introduced the method for the determination of effective electrical parameters for ECT from a systematic *in vitro* study performed on cells in culture (Maček-Lebar et al., 2002; Larkin et al., 2007). It has been proven that ECT parameters optimized *in vitro* are applicable *in vivo*. Currently, eight or two groups of four 100 µs square wave pulses with a repetition frequency of 1 Hz or 5 kHz are most commonly used. In the ESOPE clinical study, a 5-kHz electric pulse repetition frequency was used based on preliminary data assuming that higher electric pulse frequency has a comparable effect as lower pulse repetition frequency in ECT (Marty et al., 2006). The advantages of a higher frequency are shorter duration of electroporation, the sensation of only one application of electric pulses and also muscle contraction is obtained only right after the electric pulses, delivery, therefore an electrode displacement due to muscle contraction during pulse' delivery is avoided. Patients report less pain is associated with 5 kHz than with 1-Hz repletion frequency electroporation (Županič et al., 2007; Serša et al., 2010). Pulse voltage amplitude is most commonly somewhere between 200 and 1000 V. It is electrode and target tissue dependent, which means it should be set to the value that ensures the electric field between the electrodes is higher than 400 V/cm. From Table 12.1 various implementations of electrodes and associated voltage amplitudes can be observed. Within a Cliniporator project, a clinical electroporator was designed, which is classified as a medical device and it is for now the only one with a medical device CE mark. SOP bases on the use of Cliniporator with associated electrodes, but

new electric pulse generators are coming to the market, with new electrodes that might have a completely different design, we always need to keep in mind that the voltage amplitude must be optimized specifically for each electrode configuration.

As with all treatments ECT also has some side effects. Transient lesions and some localized pain can appear in areas that are in direct contact with the electrodes (Mir and Orlowski, 1999). A problem can also occur if electroporation pulses interfere with heart muscle rhythm. There is very little chance for this phenomena when the application is used for skin treatment (Mali et al., 2008). But deep-seated tumors, which can be located close to the heart, are also treated with ECT, and even in an open surgery the probability of electroporation pulses interfering with the heart is increasing. The most dangerous possible interference is induction of ventricular fibrillation (Wiggers and Wegria, 1940; Han, 1973; Reilly, 1998). Fibrillation can occur if the amplitude of the applied electric pulses is greater than the threshold level for fibrillation, and if electrical pulses are delivered during late atrial or ventricular systole. The vulnerable period for ventricles is near the peak of the T wave, and for atria in the S wave (Mali et al., 2005). The delivery of electric pulses must be synchronized with the ECG so as to reduce the risk (Mali et al., 2015).

12.2.2 Irreversible Electroporation

IRE as a nonthermal tissue ablation is a promising application for ablation of tumors tissue that is located near bile ducts or blood vessels (Scheffer et al., 2014). IRE causes cell death due to cell membrane electroporation and not due to tissue's temperature increase; however, a local temperature increase occurs around the electrodes, when a greater number of pulses are administered. IRE has almost the same main challenges as ECT, the tumor tissue should be covered with an adequate electric field, but in case of IRE, the electric field should be above the IRE threshold (Rubinsky et al., 2007). In addition, the magnitude should be selected to minimize the electroporation of healthy tissue, so as to avoid significant thermal damage (Shafiee et al., 2009; Županič and Miklavčič, 2011). It is mainly used for the treatment of deep-seated tumors either during open surgery or percutaneously in liver, pancreas, kidney, lung, and other organs.

IRE does not have an SOP, treatment protocols vary with research groups, tumor types, and stages of development. An individual treatment plan required for each specific tumor and is crucial for successful outcome. IRE can be minimally invasive in combination with ultrasound, computed tomography guidance, or magnetic resonance imaging (Jourabchi et al., 2014). In comparison to ECT, safety is even more important, because with IRE we are ablating about 50 cm^3 of tissue and the number of applied pulses is at least 90 (Bertacchini et al., 2007). To achieve IRE threshold, applied electric fields should be higher, i.e., delivered pulses should have amplitudes up to 3000 V and currents up to 50 A (Bertacchini et al., 2007). A Cliniporator VITAE or NanoKnife electroporator is used (their specifications can be found in Table 12.2). Higher electric fields, open surgery, and proximity of the heart raises the risk, delivered pulses might interfere with cardiac activity if delivered at inappropriate heart rhythm phase (Thomson et al., 2011). Pulses should be synchronized with the refractory period of the cardiac rhythm. The overall time for the procedure is extremely short in comparison to benchmark

treatments. It lasts only a few minutes, actual time can be calculated from the number of delivered pulses and average heart rate, because pulse delivery is coupled to the heart rate (Davalos et al., 2005; Bertacchini et al., 2007; Rubinsky et al., 2008).

Traditional IRE is based on the use of a series of unipolar electric pulses, normally accompanied by a significant muscle contraction; therefore, general anesthesia and neuromuscular blocking agents are necessary to prevent muscle contraction (Rubinsky et al., 2008). Researchers are investigating different techniques to minimize the contractions. According to the Golberg and Rubinsky approach, surrounding a central energized electrode with a series of grounded electrodes reduces the volume of tissue exposed to electric fields with the potential to induce contraction (Golberg and Rubinsky, 2012). This procedure requires that at least 16 grounded electrodes be surrounded by one superficially inserted, energized electrode. Arena (Arena et al., 2011) uses high-frequency IRE named H-FIRE. H-FIRE utilizes high frequency, bipolar bursts to eliminate muscle contraction, without sacrificing the efficiency of cell death due to nonthermal electroporation. He showed that H-FIRE at 250 or 500 kHz has the same ablation and precision outcomes as traditional IRE (Arena et al., 2011)

Treatment plans have been developed that can help clinicians. Electrode configuration and pulse parameters are proposed, but a proper electrode placement can be in some cases really challenging (Edd and Davalos, 2007; Kos et al., 2015). Clinical studies are going on all around the world trying to specify the optimal parameters for specific cancer types. Each set of pulse specifications, number of pulses, voltage amplitude, and pulse duration have an effect on IRE outcomes. Pulse length is responsible for thermal effects in tissue. The maximal duration can be calculated for each electric field that would not induce thermal effects or at least minimize them. Typical IRE pulses consist of a series of 100-μs pulses separated by at least 100 μs. The pause between pulses enables a cooldown. Davalos (Davalos et al., 2005) showed that the threshold for IRE in most cell types is at least 800 V/cm. Rubinsky (Rubinsky et al., 2008) proposed that for prostate cancer cells a field of 250 V/cm is sufficient with use of 90 pulses to ensure complete ablation of that region. Raffa demonstrated that performing IRE in the presence of fibril boron nitride nanotubes lowers the necessary voltage threshold required to cause tumor cell death. IRE at 800 V/cm was 2.2 times more effective at causing cell destruction when performed in the presence of fibril boron nitride nanotubes (Raffa et al., 2012). Contradictory to recent guidelines for ECT, there are no specific guidelines on how to report clinical cases and studies.

12.2.3 Gene Transfection

Gene electrotransfer is a promising non-viral gene delivery method (Kandušer et al., 2009). It is used for treatment of cancer and other diseases (Shibata et al., 2006; Daud et al., 2008), for DNA vaccination (Chiarella et al., 2010; Sardesai and Weiner, 2011), and genetic modification of organisms (Golden et al., 2007; Grewal et al., 2009). "Cancer immunoediting" is a process combining the immune system and tumors. The immune system can protect the host against tumor growth, or promote cancer development by selection of tumor variants with reduced immunogenicity (Zou et al., 2005). Immunotherapy can include cancer vaccines based on plasmid DNA (pDNA) vectors (Serša et al., 2015).

Electroporation is used to promote antigen, oligonucleotides, and immunomodulatory molecule delivery in to tumor tissue. They can stimulate the immune system or act on immunosuppressor genes (Serša et al., 2015). *In vitro* electric pulses are frequently used for the transfection of bacterial and eukaryotic cells. *In vivo* the technique is termed DNA electrotransfer, electrogenetherapy, or also gene electrotherapy. It has been successfully used since 1998. However, exact molecular mechanisms of DNA transport are unknown (Kandušer et al., 2009; Serša et al., 2015). DNA transfer can only be achieved by reversible electroporation, because dead cells are not able to express transferred genes (Andre et al., 2010). The DNA must be injected before electroporation; the application requires sufficiently intense electric fields, which means sufficiently long pulses should be applied, but we also need to ensure reversible electroporation. Permeabalized cell membrane should interact with the plasmid; thus a DNA–membrane complex is formed. DNA, then, with an as yet unknown process, is transferred into the cytoplasm and transported to the nucleus. In cases where the application is successful, the process is followed by gene expression (Golzio et al., 2002; Faurie et al., 2010).

Initially, we thought one of the most important mechanisms for efficient gene electrotransfer was electrophoretic movement of DNA during the pulse. A long millisecond, square, or exponential decaying pulses were used with 400–600 V/cm and up to 20 ms long (Bettan et al., 2000). Some studies showed that DNA transfection is enhanced in a combination of short HV and long low-voltage (LV) pulses. It was suggested that HV pulses are crucial for permeabilization of the cell membrane and pore formation, while LV pulses electrophoretically drag negatively charged DNA into the cell. Eight HV pulses 100 µs with amplitude 1300 V/cm followed by one longer 100 ms LV pulse 100 V/cm (Šatkauskas et al., 2002) were proposed. Further, Miklavčič's group showed that short HV pulses are not only crucial but also sufficient for successful DNA delivery, at optimal plasmid concentrations. They suggested that electrophoretic force of LV pulses is crucial in *in vivo* conditions where suboptimal plasmid concentration is the limiting factor for efficient transfection (Kandušer et al., 2009). As induced electric field is tissue dependent, it is important to define targeted tissue. In comparison to ECT, where targeted tissue is always tumor, a definition in the case of gene electrotransfer is more complicated. Electroporation parameters depend on the type of tumor antigen and target tissues, and the target cells in specific tissue are different (Serša et al., 2015). Electric pulse parameters have to be experimentally or numerically optimized for given electrodes' positions and geometry (Županič et al., 2010). Gene electrotransfer efficiency is electroporation media dependent, divalent cations such as Ca^{2+} and Mg^{2+} are necessary for the formation of DNA–membrane complex during the pulses. They act as a bridge between negatively charged DNA and the negatively charged cell plasma membrane, and thus improve DNA–membrane binding (Haberl et al., 2013a).

Classical gene electrotransfection parameters are hard to define; for example, 8×5 ms, 700 V/cm, 1 Hz are efficient *in vitro*. Studies show that more than 30% of cells can express the gene coded by plasmid DNA, while preserving cell viability to a large extent (Golzio et al., 2002; Chopinet et al., 2012). In the case of skin tumors, rate significantly decreases *in vivo* (Rols and Teissié, 1998; Čemazar et al., 2009). A lot of studies have been performed and parameters described to enable better gene transfer. In other studies, electric field direction and orientation changes during the pulse delivery have

been shown to increase the area, making DNA entry into the cell more competent. The introduction of DNA only occurs in the part of the membrane facing the cathode. It was shown that the percentage of cells expressing genes increases when electric field direction and orientation change (Pavlin et al., 2011). Also, a new prospect was presented, involving nanosecond electric pulses (pulse duration: 4–600 ns). Very short HV pulses (several tens of kV/cm) are able to disturb membranes of internal organelles, due to cell membranes charging time. We can conclude there is an option short nanosecond pulses can effect the nuclear envelope. Combination of medium or long electrical pulses with short HV nanosecond pulses enhance gene expression by increasing the number of plasmids entering the nucleus (Beebe et al., 2003; Chopinet et al., 2013; Guo et al., 2014).

Overall, we can say that the field of gene electrotransfer is complex and many known and as yet unknown factors mutually affect the process.

Longer electric pulses are optimal for higher transfection efficiency, but they reduce viability. Shorter pulses enable lower transfection efficiency and preserve viability. The number of studies is increasing, fields and applications are spreading, with insufficient and incomplete data and pulse parameters. There are no standard procedures or reporting guidelines defined that would in the future enable a proof of concept. In comparison to ECT, a greater variety of pulses are being used, for each tissue specific pulse parameters are optimal, for each application a specific procedure seems to be required. One of the problems is that electroporation device manufacturers produce devices with preprogramed procedures. Users only select the "appropriate program" and the device ensures optimal transfection. Pulse parameters and characteristics stay unknown due to the device patent. The field is getting more and more chaotic and is in need of a more consistent, explicit, and well-defined research with guidelines on reporting; including electric pulse protocols.

12.3 Conclusion

Electroporation is a platform technology, which is already established in medicine and food processing. When we are dealing with electroporation, measuring is crucial for achieving effective electroporation. Quality assurance can only be provided by appropriate measurements, i.e., measuring the voltage and current using an oscilloscope.

Due to the huge variation in biological load characteristics, delivered pulses may significantly deviate from the pre-set. Low impedance of a load (tissue or cell suspension) requires large power/currents, which quite often leads to significant voltage drop. Protocols in which a larger number of pulses (or long pulses) are delivered can result in reduced amplitude of pulses. The delivered pulse shape repetition frequency, pulse duration, and amplitude must be always monitored. The measurement probes should be located as close to the load as possible, the oscilloscope bandwidth should be high enough, and in the case of nanosecond application, the reflections and losses must be considered.

One of the leading applications in the electroporation field is ECT that already has well-established protocols, reporting guidelines, and good research reproducibility. Unfortunately, failed efforts to confirm other published paperwork are increasing (Kaiser, 2016). We believe the main reason for this situation is flawed descriptions of the

TABLE 12.1 Various Implementations of Electrodes, with Associated Voltage Amplitudes, Provided by the Manufacturers

Producer	Device	Type of electrodes	Number of output channels	Number of electrodes	Electrodes' geometric description		Pulse number	Pulses amplitude
Angiodynamics								
	NanoKnife	Needle	/	1–6 Probe outputs	Probes spaced 1.5 cm apart with the active electrode length set at 2 cm		90 (Pulses for each pair of electrodes)	(100–3000) V
Bionmed Technologies								
	SENNEX	Needle/pin surface	/	4	Linear layout for small tumors and pentagon layout for larger, more extensive tumors. Pin electrodes are 3 mm thick at the top and needle 0.3 mm.		8	1000 V
IGEA								
	Cliniporator EPS02	Needle	7	7	Hexagonal configuration (diameter: 0.7 mm/length: 10 mm/20 mm/30 mm)		HV: 4	HV: 730 V
		Needle	2	8 (2 × 4)	Linear configuration (diameter: 0.7 mm/length: 10 mm/20 mm/30 mm)		HV: 8	HV: 400 V
		Plate	2	2	Linear configuration (10 mm × 30 mm × 0.8 mm)		HV: 8	HV: 960 V
		Needle	2	6 (2 × 3)	Finger configuration with orthogonal linear needles (diameter: 0.7 mm/length: 5 mm/10 mm)		HV: 8	HV: 400 V

(*Continued*)

TABLE 12.1 (*Continued*) Various Implementations of Electrodes, with Associated Voltage Amplitudes, Provided by the Manufacturers

Producer	Device	Type of electrodes	Number of output channels	Number of electrodes	Electrodes geometric description		Pulse number	Pulses amplitude
		Needle	2	6 (2 × 3)	Finger configuration with longitudinal linear needles (diameter: 0.7 mm/length: 5 mm/10 mm)		HV: 8	HV: 400 V
		Partially isolated needles	7	7	Hexagonal configuration (diameter: 0.7 mm/length: 40 mm)		HV: 4	HV: 730 V
	Cliniporator VITAE	Needle	2–6	2–6	Single long needle/diameter: 1.2 mm/active part: 1–4 cm; soft tissues custom geometry ECT		HV: 4 + 4 (polarity exchange)	HV: (500–3000) V
		Needle	2–6	2–6	Single long needle/diameter: 1.8 mm/active part 1–4 cm; bones custom geometry ECT		HV: 4 + 4 (polarity exchange)	HV: (500–3000) V
Intracel								
	TSS20 Ovodyne	Silver, tungsten, and platinum electrodes	/	2	Electrodes. Silver electrodes are available made from 0.8 mm diameter silicon rubber insulated silver wire, the exposed pole being flattened into a "paddle" shape approximately 2 × 1 mm. Silver wire length extending beyond the electrode holder is 40 mm. Tungsten electrodes are produced from 0.5 mm o.d. tungsten rod		/	/

(*Continued*)

TABLE 12.1 (*Continued*) Various Implementations of Electrodes, with Associated Voltage Amplitudes, Provided by the Manufacturers

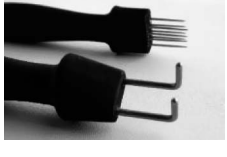
Producer	Device	Type of electrodes	Number of output channels	Number of electrodes	Electrodes geometric description	Pulse number	Pulses amplitude
Inovio							
	CELLECTRA 5PSP	Needle electrodes, intramuscular	/	5	An array consisting of 5 needle electrodes, adjustable from 13, 19, and 25 mm in length depending on BMI, forming a pentagon on a 1-cm circle	3	Max 200 V
	CELLECTRA 2000–5P	Needle electrodes, intramuscular	/	5	An array consisting of 5 needle electrodes, adjustable from 13, 19, and 25 mm in length depending on BMI, forming a pentagon on a 1-cm circle	3	Max 200 V
	CELLECTRA 2000–3P	Needle electrodes, intradermal	/	5	An array consisting of 3 needle electrodes, 3 mm in length, forming an isosceles triangle, 3-mm spacing (short side) and 5-mm spacing (long sides)	2 Sets of 2 pulses	Max 200 V
Leroy BIOTECH							
	ELECTROvet S13		/		All are compatible with plate and needle electrodes		
	ELECTROvet EZ ELECTRO cell B10		/	8 (2 × 4)	Needle electrodes are specially designed and manufactured for the treatment of subcutaneous tumors. 8 mm between the two rows of four needles. Each needle spaced 2 mm apart		
	ELECTRO cell S20 MILLIPULSES		/	2	10-mm length/10-mm spacing between electrodes centers/3 mm		

TABLE 12.2 A Review of Commercially Available Electroporation Devices, Including Their Capabilities and Characteristics

Producer	Device	Pulse description	Pulse number	Pulses amplitude	Pulses duration	Pulses repetition frequency	Pulse sequences	Maximum voltage	Maximum current
Angiodynamics									
	NanoKnife	Square wave	90 (Pulses for each pair of electrodes)	(100–3000) V	(20–100) µs	ECG synchronized, 90, 120, 240 ppm	/	3000 V	50 A
BEX Co., Ltd.									
	CUY21EDIT	Square wave	/	(1–500) V	T = 0.1–999.9 ms	/	/	/	I < 5.0 A (1–125 V) I < 2.2 A (126–250 V) I < 1.0 A (251–500 V)
	CUY21EDIT II	Square wave exponential	/	(1–200) V (Square) (1–400) V (Exponential; PP) (1–350) V (Exponential; DP)	T = 0.05–1000 ms (Square) T = 0.01–99.9 ms (Exponential)	/	/	/	I < 1 A (Square) I < 10 A (Exponential)
	Genome editor	Square wave	/	(1–200) V	T = 0.10–1000 ms	/	/	/	
	CUY21Vitro-EX	Exponential	/	1–900 V (PP) 1–500 V (DP)	T = 0.01–99.9 ms	/	/	/	I < 50 A
	LF301	Square wave sinus (AC)	/	0–1200 (Square wave) 0–75 V_{rms} (AC)	T = 0–100 µs (Square) fAC = 1 MHz; T = 0–100 s (Prefusion)/0–10 s (Post-fusion)	/	/	/	R > 50 Ω

(*Continued*)

TABLE 12.2 (*Continued*) A Review of Commercially Available Electroporation Devices, Including Their Capabilities and Characteristics

Producer	Device	Pulse description	Pulse number	Pulses amplitude	Pulses duration	Pulses repetition frequency	Pulse sequences	Maximum voltage	Maximum current
Bionmed Technologies									
	SENNEX	Square wave	8	1000 V	100 µs	100 ms	10 Imp/s	1000 V	/
BTX-Harvard Apparatus									
	AgilePulse *in vivo* system	/	3 Groups of pulses: from 1 to 10 pulses in each group	(50–1000) V	(0.050–10) ms	(0.200–1000) ms	/	1000 V	At max voltage and minimum resistance: 1000 V/10 Ω = 100 A
	AgilePulse MAX system	/	3 Groups of pulses: from 1 to 10 pulses in each group	(50–1200) V	HV: (0.050–10) ms	(0.200–1000) ms	(1–5000) Hz	1200 V	At max voltage and minimum resistance: 1200 V/10 Ω = 120 A
	ECM 2001	Square wave	1–9	HV: (10–3000) V LV: (10–500) V	HV: (1–99) µs LV: (0.01–0.99) ms	(0.01–0.99) ms (1–99) ms		3000 V	/
		AC		(0–150) V (Vpp)	Duration: (0–99) s	Post fusion—ramp: 1–9 s	1 mHz		
	ECM 830	Square wave	1–99	HV: (505–3000) V LV: (5–500) V	HV: (10–600) µs LV: (10–999) µs; (1–999) ms; (1–10) s	100 ms–10 s	/	3000 V	500 A limit at 100 µs
	ECM 630	Exponential decay wave	1–99	HV: (50–2500) V LV: (10–500) V	10 µs–10 s	/	/	2500 V	6000 A in LV mode
	Gemini SC2	Square waves and exponential decay waves	LV: 1–10 HV: 1–2 Exponential decay: 1	(10–3000) V	50 µs–100 ms	100 ms–30 s	/	3000 V	/

(*Continued*)

TABLE 12.2 (*Continued*) A Review of Commercially Available Electroporation Devices, Including Their Capabilities and Characteristics

Producer	Device	Pulse description	Pulse number	Pulses amplitude	Pulses duration	Pulses repetition frequency	Pulse sequences	Maximum voltage	Maximum current
	Gemini X2	Square waves and exponential decay waves	Square wave: LV mode-1–120 (10 per sample) HV mode-1–36 (3 per sample); Exponential decay: 1–12 (R internal <100 Ω) and 1–24 (R internal > 100 Ω)	(5–3000) V	10 µs–1 s	100 ms–30 s	/	3000 V	/
	ECM 399	Exponential decay waves	1	(2–2500) V	Max. at 500 V: 125 ms; Max. at 2500 V: 5 ms	100 ms–10 s	/	2500 V	/
Cyto Pulse Science, Inc.									
	OncoVet	/	/	(50–1000) V	(0.05–10) ms	(0.2–1000) ms	(1–5000) Hz	/	/
IGEA									
	Cliniporator EPS02	Square wave	LV: 1–10 HV: 1–10	LV: (20–200) V HV: (100–1000) V	LV: (1–200) ms HV: (50–1000) µs	LV: (0.45–500) Hz HV: (1–5000) Hz	24 Configurations	1000 V	LV: 5 A HV: 20 A
	Cliniporator VITAE	Square wave	HV: 4 + 4 (polarity exchange); 4–8	HV: (500–3000) V	100 µs	HV: (1–5000) Hz	Costum	3000 V	50 A
Inovio									
	CELLECTRA 5PSP	Square wave	3	Max 200 V	52 ms	1 Hz	/	Max 200 V	0.5 A Constant current

(*Continued*)

TABLE 12.2 (*Continued*) A Review of Commercially Available Electroporation Devices, Including Their Capabilities and Characteristics

Producer	Device	Pulse description	Pulse number	Pulses amplitude	Pulses duration	Pulses repetition frequency	Pulse sequences	Maximum voltage	Maximum current
	CELLECTRA 2000–5P	Square wave	3	Max 200 V	52 ms	1 Hz	/	Max 200 V	0.5 A Constant current
	CELLECTRA 2000–3P	Square wave	2 Sets of 2 pulses	Max 200 V	52 ms	3 Hz	2 Sets seperated by 3 s	Max 200 V	0.2 A Constant current
Intracel									
	TSS20 Ovodyne	Square wave	1–999	(0.1–99.9) V	(1–999) ms	(10–9990) ms	1–999	99.9 V	100 mA TSS20 1000 mA EP21
Leroy Biotech									
	ELECTROvet S13	Square wave pulse generator	1–10,000 or infinite	0–1350 V	5–5000 µs	0.1–10,000 ms	0.1–10,000 Hz	1300 V	10 A
	ELECTROvet EZ	Square wave pulse generator	1–10,000 or infinite	0–1500 V	5–5000 µs	0.1–10,000 ms	0.1–10,000 Hz	1500 V	25 A
	ELECTRO cell B10	Square wave bipolar pulse generator—high voltage low voltage	1–10,000 or infinite	0–1000 V	5–5000 µs	0.1–10,000 ms	0.1–10,000 Hz	1000 V	10 A
	ELECTRO cell S20	Square wave pulse generator	1–10,000 or infinite	0–2000 V	5–5000 µs	0.1–10,000 ms	0.1–10,000 Hz	2000 V	25 A

(*Continued*)

TABLE 12.2 (*Continued*) A Review of Commercially Available Electroporation Devices, Including Their Capabilities and Characteristics

Producer	Device	Pulse description	Pulse number	Pulses amplitude	Pulses duration	Pulses repetition frequency	Pulse sequences	Maximum voltage	Maximum current
Molecular Devices									
	Axoporator 800 A	Square and bi-level pulses with positive and negative polarity, as well as bipolar	Train duration: 10 ms–100 s	±(1–100) V	MONOPOLAR: 200 µs–1 s BI-POLAR: 400 µs–1 s BI-LEVEL: 10 ms–20 s	MONOPOLAR ftrain = (1–2000) Hz BI-POLAR ftrain = (1–2000) Hz BI-LEVEL ftrain = (0.024–50) Hz	Rectangular pulse Bipolar pulse Postive bi-level pulse	5 V peak to peak	±10.0 µA
NPI									
	ELP-01D	Square wave	/	(0–110) V	(0–9999.9) ms	(0–9999.9) ms	/	/	/
OnkoDisruptor									
	Onkodisruptor Electroporator	/	/	/	/	50 + 50 µs Pause: 10 µs s	8 Biphasic	1500 V	5 A
Supertech Instruments									
	SP-4a	With RC time constant of the exponential decay of wave	0–99	HV: (200–400) V LV: (0–200) V	(1–250) ms	(10–60,000) ms	Single pulse mode and burst mode (up to 99 pulses)	400 V	11.9 A

Sources: Angiodynamics, http://www.angiodynamics.com/; BEX Co., Ltd., http://www.bexnet.co.jp/; Bionmed Technologies, http://bionmed.de/; BTX-Harvard Apparatus, http://www.btxonline.com; Cyto Pulse Science, Inc., http://www.cytopulse.com/; IGEA, http://www.igea.it/; Inovio, http://www.inovio.com/; Intracel, http://intracel.co.uk/; Leroy Biotech, https://www.leroybiotech.com; Molecular Devices, http://www.moleculardevices.com/; NPI http://www.npielectronic.de/; OnkoDisruptor, https://www.onkodisruptor.com; Supertech Instruments, http://www.superte.ch/Electroporator.html

Note: We wrote to all listed producers and kindly asked them to review the data we found in literature and on the Internet and update if necessary. While most of the manufacturers were pleased to cooperate, we did not get information about pulse characteristics from others. We are still missing all the information about pulse characteristics from, Lonza, TriTech, Ichor Medical Systems, Thermo Fischer, Eppendorf, Maxcyte, and Oncosec.

equipment used and the process. Many papers describing/using electroporation have reported insufficient details, and quite often measurements are not reported (Batista Napotnik et al., 2016; Campana et al., 2016). The field of electroporation is in need of promoting reproducible research that can only be achieved by adequate measurements, standardized reports, and proper use of electroporators and electrodes.

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