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Concepts of Electroporation Pulse Generation and Overview of Electric Pulse Generators for Cell and Tissue Electroporation

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16.1 Introduction

It is well accepted now that the efficacy of electroporation, a phenomenon that occurs in lipid membranes exposed to strong electric fields, depends on several physical and biological parameters (Wong and Neumann, 1982; Mir, 2001; Rols, 2006). These are parameters of the electric field (i.e., pulse amplitude, pulse duration, number of pulses, pulse repetition frequency, pulse shape, and electric field direction) (Rols and Teissie, 1998; Vernhes et al., 1999; Kotnik et al., 2001a,b, 2003; Macek-Lebar and Miklavcic, 2001; Canatella et al., 2001; Pucihar et al., 2002; Faurie et al., 2004; Rebersek et al., 2007) and cell parameters that define the state of cells, their surroundings, and cell geometry (i.e., cell size and shape, temperature, osmotic pressure, etc.) (Rols and Teissie 1992; Rols et al. 1994; Kotnik et al., 1997; Bobrowska-Hagerstrand et al., 1998; Golzio et al., 1998; Pucihar et al., 2001). Cell parameters are diverse and usually cannot be controlled. Therefore, in electroporation applications, the parameters of the electroporation signal are optimized to specific cells, tissues, and most of all to achieving electroporation objectives. For example, in DNA transfection, the pulse amplitude is optimized to the specific cell size to achieve reversible cell membrane electroporation and to the pulse duration to allow plasmid DNA membrane complex formation, which then leads to gene expression. Electroporation can be reversible or irreversible (Figure 16.1), where reversibility/irreversibility is related to cell survival/death. Reversible

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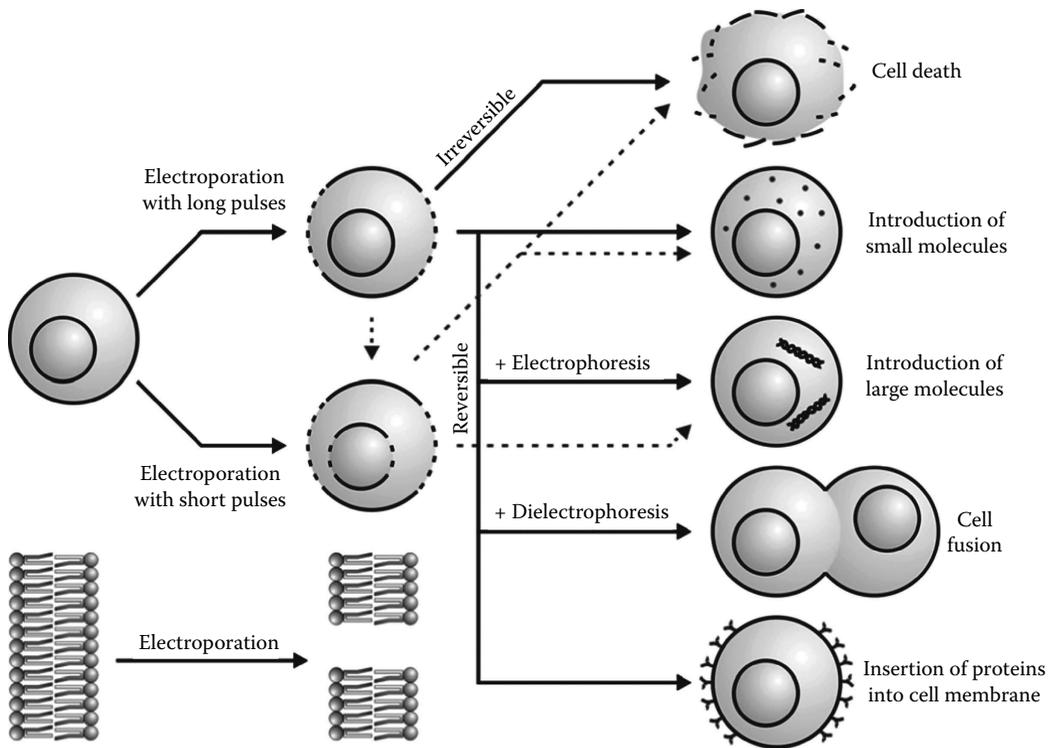
FIGURE 16.1 Electroporation of a cell may be reversible or irreversible. Reversible electroporation is characterized by cell survival and can be even furthermore optimized for introduction of small and large molecules, fusion of cells, and insertion of proteins into cell membrane. For some of these specific applications an auxiliary pulses are sometimes used such as electrophoretic and dielectrophoretic pulses. Electroporation may also be used on membrane model systems such as planar lipid bilayer and vesicles.

electroporation can be even further optimized for specific objectives, for example, the introduction of small and large molecules, the fusion of cells, and the insertion of proteins into the cell membrane. At this optimization, auxiliary pulses are sometimes used, such as electrophoretic pulses for DNA and dielectrophoretic pulses for cell pearl chain formation in fusion.

Nowadays, electroporation is widely used in various biological, medical, and biotechnological applications (Table 16.1) (Kanduser and Miklavcic, 2008). It is used for electrochemotherapy (Mir et al., 1995,

TABLE 16.1 Pulse Parameters for Different Electroporation Applications

Application	Amplitude	Duration	Auxiliary Pulses
Electrochemotherapy	~kV	μs, usually $8 \times 100\,\mu s$	—
Gene electrotherapy	~kV	μs–ms	Electrophoretic pulses <500 V, >ms
Electroinsertion	<kV	ms–s	—
Transdermal drug delivery	<kV	ms	—
Electrofusion	~kV	μs	Dielectrophoretic pulses <200 V, >s, ~MHz
Pasteurization	≫kV	μs	—
Tissue ablation	>kV	μs–ms	—
Single cell electroporation	>mV	μs	—
Electroporation research	mV–kV	ns–s	—

2006; Heller et al., 1999; Rols et al., 2002; Sersa, 2006), gene electrotransfer (Ferber, 2001; Mir, 2001; Satkauskas et al., 2002; Herweijer and Wolff, 2003; Prud'homme et al., 2006), cell fusion (Zimmermann, 1982; Ramos and Teissie, 2000; Trontelj et al., 2008), insertion of proteins into cell membranes (Mouneimne et al., 1989), transdermal drug delivery (Vanbever et al., 1994; Pavselj and Preat 2005), water treatment (Teissie et al., 2002), food preservation (Beveridge et al., 2002), tissue ablation (Rubinsky et al. 2007), and electroporation of cell organelles (Schoenbach et al., 2001; Rebersek et al., 2009). With microelectrodes, electroporation can be used on a single cell (Agarwal et al., 2009). Electroporation is sometimes also used or studied on simpler lipid membranes such as planar lipid bilayers or multilayers, vesicles, etc. (Montal and Mueller, 1972; Dimova et al., 2009; Kramar et al., 2009). Pulse amplitude is lower in the case of planar bilayer membranes and higher in the case of vesicles.

The diversity of the electrodes and target cells (either single cells, cell suspensions *in vitro*, or cells in tissues *in vivo*) is vast and only a rough introduction of the most important parameters of the electric field can be given. Pulse amplitudes that are used for reversible electroporation at electric pulse durations of $100\,\mu s$ vary from a few V up to a few 100 V, for irreversible electroporation they vary from a few 100 V to several kV, and for electroporation of lipid bilayers they vary from 10 mV to V (Figure 16.2a).
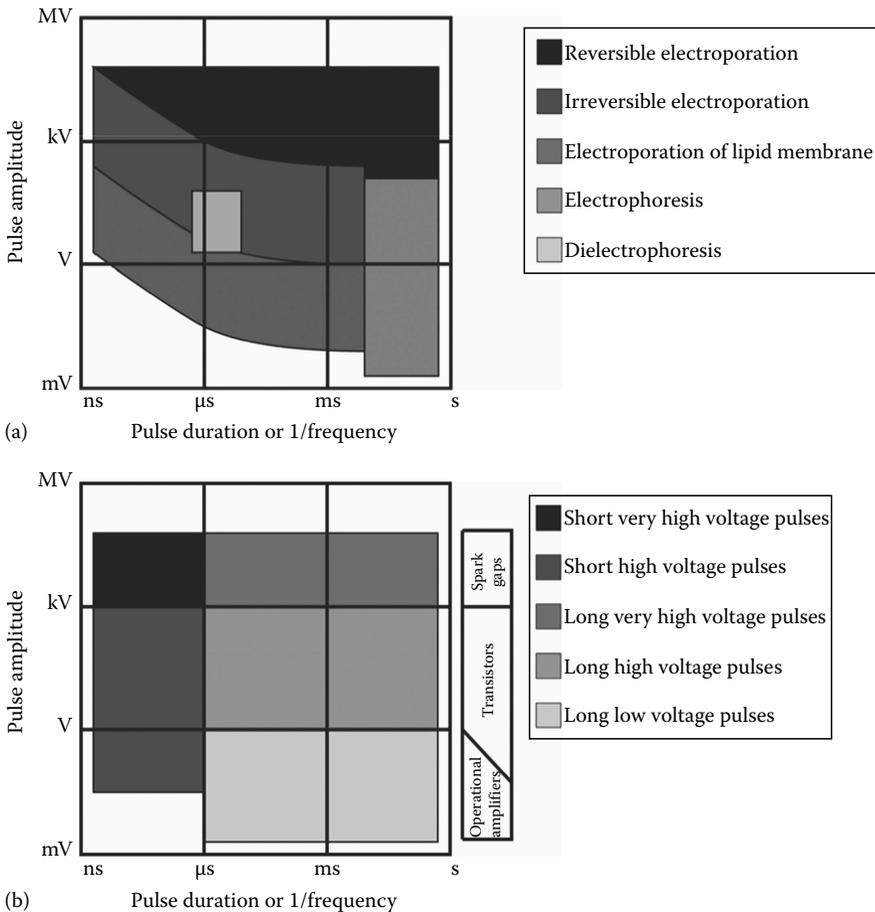
FIGURE 16.2 Areas of amplitude and duration of electrical pulses which are used in the research of electroporation and related effects (a). Five different areas of electroporation pulse generation (b). To amplify or to generate very-high-voltage electroporation pulses (over a few kV) spark gaps and similar elements are used, for high-voltage (a few V to a few kV) transistors and for low-voltage operational amplifiers are used. Nanosecond (short) pulses are generated with different techniques than pulses longer than $1\,\mu s$.

For shorter pulses, mainly in the nanosecond range, much higher amplitudes have to be used because of the lipid membrane time constant. On the other hand, for longer pulses, slightly lower amplitudes have to be used for a comparable electroporation effect. The auxiliary signals for gene electrotransfer and cell fusion, namely electrophoretic and dielectrophoretic pulses, have considerably lower amplitudes from the ones needed for electroporation. Electrophoretic pulses are at least a few milliseconds long and dielectrophoretic pulses are in the frequency range from a few 100 kHz to a few MHz. In summary, electroporation pulses (depending on the application) have amplitudes ranging from mV to kV and with frequency content from Hz to GHz. Such a variety of signals/pulses can evidently not be generated by a single generator.

16.2 Concepts of Electroporation Pulse Generation

The effectiveness of electroporation depends on the distribution of electric fields inside the treated sample (Sersa et al., 1996; Miklavcic et al., 1998; Gehl et al., 1999). To achieve (effective) electroporation, we have to use an appropriate set of electrodes (e.g., needle, parallel plates, cuvettes, etc.) and an electroporation device—an electroporator that generates the required voltage or current signals. Although both parts of the mentioned equipment are important for the effectiveness of electroporation, the electroporator is a more complicated device as it has to be able to generate the required signal and deliver the necessary current/power, which is defined by the load (Puc et al., 2001; Pavselj and Miklavcic, 2008).

To design or purchase an electroporator, one has to know the application for which the electroporator will be used. For example, clinical devices have to meet medical safety standards [IEC-60601], gene electrotransfer requires high- and low-voltage pulses (Satkauskas et al., 2002; Kanduser et al., 2009), cell electrofusion requires dielectrophoretical pulses (Pilwat et al., 1981), multi-needle electrodes require electrode commutators (Rebersek et al., 2007), etc. However, it is also important to know the range of electrical parameters that will be used. The maximal voltage and current of electroporation pulses define the switching elements that will be used in the design, because a particular switching element works as required only in the specific voltage and current range. For very high-voltage electroporation pulses (over a few kV), spark gaps and similar elements are used; for high-voltage electroporation pulses (from several V to a few kV), transistors are used; and for low-voltage electroporation pulses (below several V), operational amplifiers are used (Figure 16.2b).

The next parameter that has a crucial role in electroporation is the duration of the electroporation pulses. At pulses shorter than 1 μs, the rise time of the pulse is usually shorter than the electrical length between the source and the load. Therefore, it is very important to match the impedance of the load to the impedance of the generator, so that there are no strong pulse reflections and consequently pulse prolongations. However, for pulses longer than 1 μs, it is much more important to consider the fact that the load impedance varies during the pulse or sample-to-sample and that the load conduction is not predefined. Generators of nanosecond pulses are thus considerably different than for classical electroporation and are described in this book (Kolb, 2010). Another important parameter to be considered is the pulse shape. For very-high-voltage pulses, only exponential and square wave pulses can be used, as very-high-voltage switches work discretely. However, for lower voltages, arbitrary pulses can be generated as switches at these voltages can also operate linearly (Flisar et al., 2003). There are a variety of different concepts of signal generation for electroporation available. Each concept allows for the generation of different pulse shapes and has specific advantages and disadvantages and is thus more or less appropriate for specific electroporation applications (Table 16.2). Signal generators for electroporation can be divided into four major groups: capacitor discharge, square wave generators, modular square wave generators, and analog generators. For special applications, electroporators can be upgraded with additional modules, for example, low-voltage modules, dielectrophoretic modules, and a commutator.

TABLE 16.2 Comparison of Different Concepts of Signal Generation for Electroporation

Concept	Application	Advantages	Disadvantages
Capacitor discharge	Bacterial and yeast gene electrotransfer	Simple and inexpensive construction Simple control system High voltages	Poor flexibility and control of parameters Low cell survival
Square wave generators	Tissue ablation, electrochemotherapy, electroinsertion	Simple control system High currents Good control and flexibility of time parameters	Amplitude drop during the pulse Low amplitude flexibility
Modular square wave generators	Electrochemotherapy, gene electrotherapy, electroporation control, electroporation research	Wide flexibility of pulse parameters Arbitrary signal shape Electroporation control High currents	Limited amplitude resolution Complex control system Price
Analog generators	Electrochemotherapy, gene electrotherapy, electroporation control, electroporation research	Wide flexibility of pulse parameters Arbitrary signal shape Electroporation control	Complex control system Limitation of power dissipation
Additional low-voltage module	Gene electrotherapy, transdermal drug delivery	Electrophoretic drag Wide flexibility of amplitude and pulse duration	Complex control system
Additional dielectrophoretic module	Cell fusion	Pearl chain formation	Complex control system
Additional commutator	Large tumor treatment, gene electrotransfer, cell fusion	Multi-needle electrodes Polarity control Electric field direction control Higher safety	Complex user interface and control system

16.2.1 Capacitor Discharge

This is the oldest concept of electroporation pulse generation primarily used *in vitro* but also *in vivo* (Neumann and Rosenhec, 1972; Okino and Mohri, 1987). The concept comprises a variable high-voltage power supply (V), a capacitor (C), a switch (S), and optionally a resistor (R) (Figure 16.3). The generator operates in two phases, charge and discharge, and generates exponentially decaying pulses. During the charge phase, the switch (S) is in position 1 and the variable high-voltage power supply (V) charges the capacitor (C) to the preset voltage. In the discharge phase, the switch is in position 2 and the
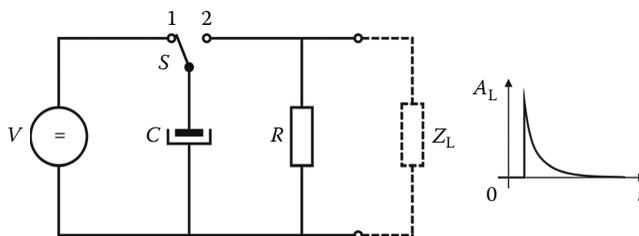
FIGURE 16.3 Capacitor discharge circuit for generation of exponentially decaying pulses. The concept comprises a variable high-voltage power supply (V), a capacitor (C), a switch (S), and optionally an internal resistor (R). The generator operates in two phases, charge (switch is in position 1 and capacitor charges to the preset voltage) and discharge (switch is in position 2 capacitor discharges through the load connected to the electrodes).

capacitor discharges through the load connected to the output. The time constant of discharge τ can be approximated by the product $Z_L C$, where C is the capacitance of the capacitor and Z_L is the absolute value of the load impedance. However, the impedance of biological load reduces during the pulse delivery (Pavlin et al., 2002, 2005; Davalos and Rubinsky, 2004; Granot et al., 2009). This also means that the time constant changes during the pulse. Therefore, most commercially available capacitor discharge-based electroporators have built-in resistances that are connected in parallel to the load. Their main purpose is to better define the time constant of the discharge. Namely, if additional resistors are connected in parallel to the load, the time constant of discharge is defined by $(R \| Z_L)C$, where R is the resistance of the internal resistors. If the absolute value of the impedance of load Z_L is at least 10 times larger than the resistance R ($Z_L \geq 10R$), the time constant can be approximated by the RC product (Equation 16.1):

$$(R \| Z_L)C = \left(\frac{RZ_L}{R + Z_L} \right)C = \left(\frac{10R}{11R} \right)C \cong RC; \quad (Z_L = 10R) \tag{16.1}$$

The presented capacitor discharge concept is very simple and inexpensive for construction. The exponentially decaying pulse generated can be used even for gene transfection as it includes the high-voltage part for permeabilization and low-voltage electrophoretic part (Satkauskas et al., 2002; Kanduser et al., 2009). However, the flexibility of such high- and low-voltage pulse composition is very poor, as the electrical parameters of the high-voltage part cannot be changed without affecting the low-voltage part and vice versa. Moreover, the low-voltage part is usually undesired in other electroporation applications, as it mainly affects the cell viability (Danfelter et al., 1998). Also, the repetition frequency of such pulse generation is low due to the relatively long charge phase.

16.2.2 Square Wave Pulse Generators

For better control of electric field parameters, square wave pulse generators have been introduced. The concept is very similar to the capacitor discharge concept, except that the voltage power supply (V) constantly charges the capacitor (C) and the power switch (S) is capable of fast switching (Figure 16.4) (Tokmakci, 2006; Bertacchini et al., 2007). Usually, fast power metal oxide semiconductor field effect transistors (MOSFETS) or insulated gate bipolar transistors (IGBTs) are used as the switch. The output amplitude of the pulse is defined by the amplitude of variable power supply, while pulse duration, pulse repetition frequency, and possibly the number of pulses are defined by the switching sequence of the fast power switch. As the switching sequence is faster and more complex, the control unit of the generator must also be faster and more complex than the capacitor discharge generator.

Despite improved control over the electric field parameters, this concept still has drawbacks that limit the flexibility and accuracy of pulse parameters available to the user. The main problem is that
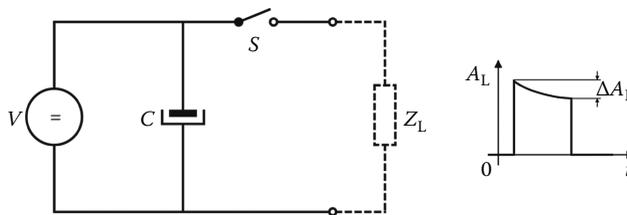

FIGURE 16.4 High-voltage power supply switching circuit for generation of square wave pulses. The concept comprises a variable high-voltage power supply (V), a capacitor (C), and a fast switch (S). The variable high-voltage power supply continuously charges the capacitor that stores energy required during the pulse. To deliver the pulse to load, the fast switch is turned on for the duration of the square wave pulse.

electroporation pulses have high power but are very short. Thus, a power supply cannot generate the energy for the pulse during the pulse generation, but it has to be generated and stored into the capacitor before the generation of the pulse. This usually results in a voltage drop (ΔA_L) during the pulse. In order to minimize this voltage drop, a very large capacitance is needed. However, as a consequence of a very large capacitance, it is now harder to change the amplitude between the pulses. Therefore, square wave pulse generators usually generate pulses with only one (preset) voltage. Nevertheless, at very high loads (very high current flow) voltage on the capacitor will inevitably decrease during the pulse generation. As it is usually required that each pulse has the same amplitude as the first one that was generated, the next pulse can only be delivered after the capacitor is recharged to the preset voltage. Therefore, limited power supply also defines the highest pulse repetition frequency.

16.2.2.1 Modular Square Wave Pulse Generator

This concept was designed to improve the output amplitude flexibility of the square wave pulse generator. The concept was described previously in detail (Petkovsek et al., 2002). Briefly, it consists of several (N) square wave pulse generators connected in a series (Figure 16.5). The generators have galvanically insulated high-voltage power supplies (V). Each square wave pulse generator is controlled individually based on the principle of a digital-to-analog (D/A) converter, thus the amplitude of the particular source V_N is twice as high as the predecessor ($V_N = 2V_{N-1}$). The voltage of the individual generator is constant and can contribute to the generation of a common output pulse at any time. With an appropriate control of switches S_1–S_N, a total of 2^N different output voltage levels with the resolution of V_1 are obtained (Petkovsek et al., 2002). Although the design of each individual source is similar to the design of the previously described square wave pulse generator, the individual power supply used in this concept has constant (not variable) output voltage. As constant voltage power supplies do not discharge during the pulse delivery, they are simpler to be designed to sustain the maximum possible current during the pulse generation. In this way, the output amplitude does not decrease during the pulse delivery.
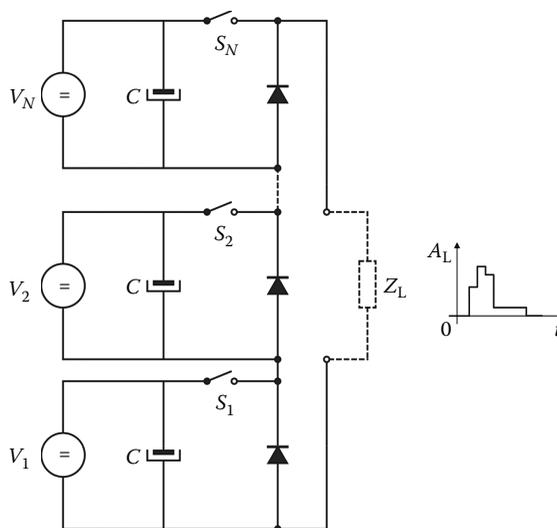
FIGURE 16.5 Modular square wave pulse generator. Operation of the device is based on a principle of D/A converter, thus the device comprises several (N) individually controlled electrically insulated DC voltage modules, where the amplitude of the particular voltage source V_N is twice as high as in the preceding module. With an appropriate control of switches S_1–S_N the modules are connected in series and a total of 2^N different output voltage levels with the resolution of V_1 are obtained.

The presented modular concept can generate well-defined pulses as its rise and fall times are fast and the amplitude is stable. However, to have enough output voltage levels, many square wave pulse generators are needed (depending on "voltage" steps/resolution), which consequently increases the cost of the device.
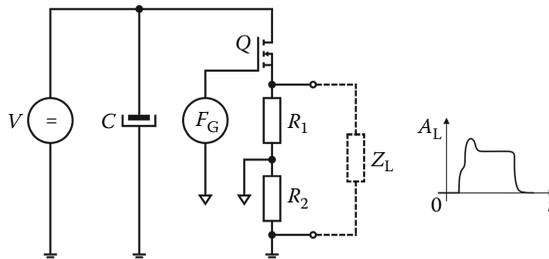
16.2.3 Analog Generators

Although square wave and exponentially decaying pulses were and probably still are the most frequently used signals for electroporation, analog generators definitely have some advantages over them. The concept of analog generators was designed to generate arbitrarily shaped electroporation pulses and to improve the output amplitude stability of the square wave pulse generator (Figure 16.6) (Petkovsek et al., 2002; Rebersek et al., 2007). The concept comprises variable high-voltage power supplies (V), capacitors (C), signal generators (F_G), linear switches (Q), and resistor decades (R_1 and R_2).

Energy for the pulse is stored in the capacitor by setting the power supply voltage higher than the maximal generated amplitude. Therefore, lower capacitance is needed to store the energy for the pulse and the amplitude of the pulse will not drop during the pulse generation, unless the capacitor voltage drops below the expected output amplitude. Usually, the preset voltage is at least 10% or 50 V higher than the maximal generated amplitude.

The pulse shape is first generated by the signal generator, which is usually a computer with a D/A converter. This signal is then amplified by a linear amplifier. Usually, an amplifier with a common source and galvanically separated input is used (Figure 16.6), as it is noninverting voltage and current amplifier. This amplifier needs a galvanic separation between the driving and the power supply circuit. This, however, is definitely not a drawback, as all electroporators should have galvanically insulated output for safety reasons. The linear amplifier consists of a linear switch (usually MOSFET or IGBT) and resistor decade. A resistor decade is used as a feedback for the regulation of the output amplitude. The signal, reduced for a voltage threshold of the linear amplifier (U_T), is therefore amplified by the factor $(R_1 + R_2)/R_1$. If the current amplification in this stage is not high enough, a current amplifier (common source) can be added to the output.

This design allows wide flexibility of all electrical parameters and electroporation control (Cukjati et al., 2007), yet some drawbacks still exist. The driving stage is much more complex than in previously described concepts and the rise and fall times of the pulses cannot be as fast as with a square wave pulse generator. Nevertheless, the major drawback of this concept is the safe operation area (SOA; voltage, current, power, and energy limitations) of linear transistors. Therefore, the duration, voltage, and current of the pulse are limited as there is high power dissipation when the transistor is working in its

FIGURE 16.6 Analog generator for generation of arbitrary pulses comprises a variable high-voltage power supply (V), a capacitor (C), a signal generator (F_G), a linear switch (Q), and a resistor decade (R_1 and R_2). Energy for the pulse is stored in the capacitor by setting the power supply voltage higher than the maximal generated amplitude. The pulse shape is first generated by the signal generator, which is usually a computer with a D/A converter. This signal is then amplified by a linear amplifier (Q, R_1, and R_2). Resistor decade is used as a feedback for regulation of the output amplitude.

linear area. Analog generators can also be designed to generate bipolar pulses by means of a push-pull amplifier (Flisar et al., 2003). However, for these generators, the current between the pulses (zero driving voltage) has to be minimized.

16.2.4 High-Voltage, Low-Voltage, and Dielectrophoretic Signals

Electroporators that are designed for gene electrotransfer ideally would have to generate high-voltage pulses for reversible electroporation and low-voltage pulses for the electrophoretic drag of DNA toward the permeabilized plasma membrane (Satkauskas et al., 2002; Kanduser et al., 2009). Electroporators that are designed for cell fusion ideally should generate high-voltage pulses for reversible electroporation and sinusoidal signals for the dielectrophoretic force (Pilwat et al., 1981). For low-voltage electroporators, these pulses can be generated by means of one analog generator. However, for high-voltage electroporators, a modular concept has to be used. A modular concept means that the electroporator is made of two or more generators, each having a special capability. Thus, an electroporator for gene electrotransfer is usually made of two square wave pulse generators. Two generators are used so that the power supply voltage of a generator does not change between the pulses and square wave pulse generators are used as they have good control over electric field parameters with respect to the expenses. Similarly, an electroporator for cell fusion is usually made of one square wave pulse generator for reversible electroporation and one bipolar analog generator for sinusoidal signal. In an electroporator designed for gene electrotransfer and cell fusion, all three modules (high voltage, low voltage, and dielectrophoretic) can be integrated into one device. The outputs of the modules are connected together with the diodes if all the output signals are unipolar or with the relays if one of the modules is generating bipolar pulses. The control of different signals using this modular approach is good, but the price of such a device can be considerable.

16.2.5 Multi-Electrode and Polarity Control

Electroporators (E_P) can be supplemented with an output commutator that can deliver electroporation pulses to multiple electrodes (E_X) and can control the polarity of the pulse (Figure 16.7). A typical commutator has an integrated one-half bridge switch for each electrode. The half bridge switch is designed with two switches, one for the positive pole (S_P) and one for the negative (S_N) pole. These two switches, however, should never be active at the same time, as they can short-circuit the electroporator. The half bridge can be used to connect the electrode with the positive or negative pole or to disconnect the electrode. Namely, if the positive switch S_P is active, a positive pole is connected to the electrode; if the negative switch S_N is active, a negative pole is connected to the electrode; and if both switches are inactive, the electrode is in a high impedance state. Bipolar pulses are used to increase the
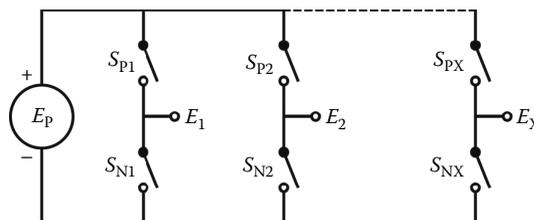
FIGURE 16.7 Multi-electrode and polarity control. Electroporators (E_P) can be supplemented with an output commutator that can deliver electroporation pulses to multiple electrodes (E_X). Usual commutator has one half bridge switch integrated for each electrode. The half bridge switch is designed of two switches, one for positive (S_P) and one for negative (S_N) pole. These two switches should however never be active at the same time, as they can short-circuit the electroporator. The half bridge can be used to connect the electrode with the positive or negative pole or to disconnect the electrode.

efficiency of electroporation (Kotnik et al., 2001a) and to reduce electrolytic contamination (Kotnik et al., 2001b). Multiple needle electrodes are used to treat larger tumors (Sel et al., 2007; Rebersek et al., 2008; Zupanic et al., 2008).

16.3 Safety

Irrespective of how simple or complex an electroporator prototype may be, safety must be the primary objective throughout their development. The most significant technical standard that should be considered during the development is the standard issued by the International Electrotechnical Commission IEC-60601, *Medical Electrical Equipment*. This standard is adopted by the European Union (EU) as EN-60601 and by the United States as UL-60601. Other countries have also adopted this standard; however, the EU and the United States are well known for their regulations that ensure medical device efficacy and safety. In the EU, a series of directives establish the requirements that manufacturers of medical devices must meet before they can obtain *CE marking* for their product to authorize their sale and use.

According to IEC-60601, a possible risk for electrical shock is present whenever a patient or operator can be exposed to a voltage exceeding $25\,V_{RMS}$ or $60\,V_{DC}$. Patient and operator safety must be ensured under both normal and single-fault conditions. Obviously, the enclosure of the device is the first barrier of protection that can protect the operator or patient from intentional or unintentional contact with these hazards. Beyond the electrical protection supplied by the enclosure, however, the circuit of the medical instrument must be designed with other safety barriers to maintain leakage currents within the limits allowed by the safety standards. The leakage current in the electroporator is minimized by the galvanical separation of electroporator output and the ground. The separation can be made in the power supply circuit or at the output of the electroporator. The galvanical separation is made by an insulation transformer for power signals or an optocoupler for data signals.

Medical devices must also meet requirements for electromagnetic interference (EMI) and electromagnetic compatibility (EMC). Therefore, electroporators should not emit electromagnetic signals that can interfere with other devices and should have sufficient immunity to operate as intended in the presence of interference (electrostatic discharge, power line transients, electromagnetic radiation, short circuit, etc.) (Prutchi and Norris, 2005).

16.4 Commercial Electroporators

Electroporation is mostly used with high-voltage electroporation pulses in the range of 100 V to 3 kV (Figure 16.2b). Reviews of commercially available electroporators were published previously (DeFrancesco, 1997; Puc et al., 2004), however, we now present an updated list of the available electroporators and their respective electrical parameters, biological applications, and signal generation techniques (Table 16.3).

Electroporators are grouped by the manufacturer and each device is presented with the following characteristics: output signal, voltage range, current (*I*)/load (*R*), time constant (τ)/pulse length (*T*), and charge time (*t*)/pulse repetition frequency (*f*). The value of the last two parameters (τ/*T* and *t*/*f*) depends on the output characteristics; if the device produces exponentially decaying pulses, the time constant and charge time are given as parameters. On the other hand, if the device generates square wave pulses, the pulse length and pulse repetition frequency are given as parameters. The data in Table 16.3 was checked and collected on the manufacturers' home pages (as written in the table) in September 2009.

Cliniporator, Cliniporator VITAE, and NanoKnife are for now the only devices approved for clinical use. CythorLab is the only device that can control the progress of electroporation, can generate arbitrarily shaped electroporation pulses, and is great in its flexibility and full control of pulse offered to the

TABLE 16.3 List of Commercially Available Electroporators with Their Parameters and Biological Applications

Company/Product	Output Characteristics	Voltage Range	Current (I)/ Load (R)	Time Constant (τ)/Pulse Length (T)	Charge Time (t)/ Pulse Repetition Frequency (f)	Biological Applications	Possible Signal Generation Technique
ADITUS MEDICAL, http://www.aditusmedical.com							
CythorLab	Arbitrary	LV: 0 V–600 V_{PP} HV: 0 V–3000 V_{PP}	NA	LV: T = up to 400 ms HV: T = up to 5 ms	NA	*In vitro, in vivo*	NA
ANGIODYNAMICS, http://www.angiodynamics.com							
NanoKnife	Square wave	100–3000 V	$I < 50$ A	T = 20–100 μs	f = 1 Hz–10 kHz	Irreversible electroporation, clinical device	Square wave
BIORAD, http://www3.bio-rad.com							
Micro Pulser	Exponential	200–3000 V	$R > 600\,\Omega$	τ = 1–5 ms	t = 5 s	Bacterial, yeast	Capacitor discharge
Gene Pulser Xcell	Exponential Square wave	10–3000 V	$R > 600\,\Omega$	τ = 0.5 ms–3.3 s T = 0.05–10 ms	t = 5 s f = 0.1–10 Hz	All cell types	Capacitor discharge
MXcell	Exponential Square wave	10–500 V	NA	τ = 1.3 ms–3.7 s T = 0.05 ms–1 s	f = 0.1–10 Hz	Eukaryotic cells	Capacitor discharge
BTX, http://www.btxonline.com							
ECM 399	Exponential	LV: 2–500 V HV: 10–2500 V	$R > 150\,\Omega$	LV: τ = 157 ms HV: τ = 5.4 ms	$t < 5$ s	All cell types	Capacitor discharge
ECM 630	Exponential	LV: 10–500 V HV: 50–2500 V	$I < 6000$ A $I < 3000$ A	LV: τ = 25 μs–5 s HV: τ = 625 μs–78 ms	$t < 5$ s	All cell types, *in vivo*, plant	Capacitor discharge
ECM 830	Square wave	LV: 5–500 V HV: 30–3000 V	$I < 500$ A	LV: T = 10 μs–10 s HV: T = 10–600 μs	f = 0.1–10 Hz	All cell types, *in vivo*, plant	Square wave generator Pulse transformer
ECM 2001	Square wave Sinus (AC)	LV: 10–500 V HV: 10–3000 V 0 V–150 V_{PP}	NA	LV: T = 10 μs–99 ms HV: T = 1–99 μs f_{AC} = 1 MHz	NA	All cell types, cell fusion	Square wave generator Pulse transformer

TABLE 16.3 (continued)   List of Commercially Available Electroporators with Their Parameters and Biological Applications

Company/Product	Output Characteristics	Voltage Range	Current (I)/ Load (R)	Time Constant (τ)/Pulse Length (T)	Charge Time (t)/ Pulse Repetition Frequency (f)	Biological Applications	Possible Signal Generation Technique
CLONAID, http://www.clonaid.com							
RMX2010	Square wave	5–200 V	NA	$T = 10$–990 µs	$f = 1$–1000 Hz	Embryonic cell fusion	Square wave generator
CYTO PULSE SCIENCES, http://www.cytopulse.com							
PA-3000	Square wave	20–1100 V	$R > 10\,\Omega$	$T = 1$ µs–10 ms	$f = 2.5$–8 Hz	*In vitro*, *in vivo*, ex vivo	Square wave generator
PA-4000	Square wave	LV: 20–400 V	$R > 10\,\Omega$	LV: $T = 1$ µs–40 ms	$f = 2$–8 Hz	*In vitro*, *in vivo*, ex vivo	Square wave generator
		HV: 20–1100 V		HV: $T = 1$ µs–1 ms			
PA-4000/PA-101	Square wave	20–800 V	$R > 10\,\Omega$	$T = 1$–100 µs	$f = 8$ Hz	Cell fusion	Square wave generator
	Sinus (AC)	15–75 V_{PP}		$f_{AC} = 0.2$–2 MHz			
PA-4000/PA-201	Square wave	LV: 20–400 V	$R > 10\,\Omega$	LV: $T = 1$ µs–40 ms	$f = 2$–8 Hz	Multi-needle electrodes	Square wave generator
		HV: 20–1100 V		HV: $T = 1$ µs–1 ms			
PA-4000/PA-301	Square wave	20–3000 V	$R > 10\,\Omega$	$T = 1$–100 µs	$f = 2.5$–8 Hz	Bacterial, yeast	Square wave generator
Derma vax	Square wave	50–1000 V	$R > 10\,\Omega$	$T = 0.05$–10 ms	$f = 1$ Hz–5 kHz	Intra-dermal	Square wave generator
Hybrimune	Square wave	100–1000 V	$R > 10\,\Omega$	$T = 20$–1000 µs	NA	Cell fusion	Square wave generator
	Sinus (AC)	10–150 V_{PP}		$f_{AC} = 0.2$–2 MHz			
CytoLVT	Square wave	50–1000 V	$R > 10\,\Omega$	$T = 0.05$–10 ms	$f = 1$ Hz–5 kHz	Large volume	Square wave generator
Onco vet	Square wave	50–1000 V	$R > 10\,\Omega$	$T = 0.05$–10 ms	$f = 1$ Hz–5 kHz	Veterinary device	Square wave generator
EPPENDORF SCIENTIFIC, http://www.eppendorf.com							
Electroporator 2510 Multiporator	Exponential	200–2500 V	$R > 600\,\Omega$	$\tau = 5$ ms	$t < 8$ s	Bacterial, yeast	Capacitor discharge
Eukaryotic module	Exponential	20–1200 V	NA	$\tau = 15$–500 µs	$t < 60$ s	Eukaryotic cells, plant	Capacitor discharge
Bacteria and yeast module	Exponential	200–2500 V	$R > 600\,\Omega$	$\tau = 5$ ms	NA	Bacterial, yeast	Capacitor discharge
Fusion module	Square wave	5–300 V	NA	$T = 15$–300 µs	$f = 1$ Hz	Cell fusion	Square wave generator
	Sinus (AC)	1–10 V_{PP}		$f_{AC} = 0.2$–2 MHz			

IGEA, http://www.igea.it

Cliniporator	Square wave	LV: 20–200 V HV: 50–1000 V	$I < 16$ A	LV: $T = 10\,\mu s$–20 ms HV: $T = 30$–200 μs	$f = 1$ Hz–10 kHz	Electrochemotherapy, gene transfection, clinical device	Analog generator
Cliniporator VITAE	Square wave	500–3000 V	$I < 50$ A	$T = 50$–1000 μs	$f = 1$ Hz–10 kHz	Electrochemotherapy clinical device	Square wave

INOVIO, http://www.inovio.com

MedPulser	Square wave	NA	NA	NA	NA	Gene transfection	NA
Elgen	Square wave	NA	NA	NA	NA	Gene transfection	NA
Cellectra	Square wave	NA	NA	NA	NA	Gene transfection	NA

LONZA, http://www.lonzabio.com

Nucleofector	NA	NA	NA	NA	NA	*In vitro* transfection	NA
CLB-Transfection	NA	NA	NA	NA	NA	*In vitro* transfection	NA

PROTECH INTERNATIONAL, http://www.protechinternational.com

CUY21EDIT	Square wave	1–500 V	$I < 5$ A	$T = 0.1$ ms–1 s	$f = 1$ Hz–10 kHz	*In vitro*, *in vivo*, ex vivo	Square wave generator
CUY21SC	Square wave	0.1–100 V	$I < 1.6$ A	$T = 0.05$–100 ms	$f = 1$ Hz–10 kHz	Single cell	Square wave generator
LF101	Square wave Sinus (AC)	0–999 V 0–60 V_{PP}	$R > 50\,\Omega$	$T = 5$–99 μs $f_{AC} = 0.1$–3.9 MHz	$f = 0.1$ Hz–10 kHz	Cell fusion	Square wave generator
LF201	Square wave Sinus (AC)	0–1200 V 0–75 V_{PP}	$R > 50\,\Omega$	$T = 1$–100 μs $f_{AC} = 1$ MHz	$f = 0.1$ Hz–10 kHz	Cell fusion	Square wave generator

TRITECH RESEARCH, http://www.tritechresearch.com

Mammo Zapper	Exponential	NA	NA	NA	$t = 15$ s[a]	Mammalian	Capacitor discharge
Bacto Zapper	Exponential	<2000 V[a]	NA	$\tau = 10$ ms[a]	$t = 5$ s[a]	Bacterial	Capacitor discharge

Source: Puc, M. et al., *Bioelectrochemistry*, 64, 113, 2004.

Note: NA, not available.

[a] Data was not available at the time this chapter was written so it has not been rechecked but has been taken from Puc et al. (2004).

experimenter. ECM 2001, PA - 4000/PA – 101, Hybrimune, Multiporator: Fusion module, LF101, and LF201 are designed to also generate a dielectrophoretic signal for cell fusion. Although the prices are not readily available and vary considerably between models and producers, they range from €10,000 to €150,000.

16.5 Conclusion

The choice of an electroporator is always driven by the objectives and application. These define the requirements for electric pulse parameters (i.e., pulse amplitude, pulse duration, number of pulses, pulse repetition frequency, pulse shape, and electric field direction). However, for electroporation research, it is very useful to have wide range, flexibility, and control over pulse parameters; therefore, such electroporators are expensive and not easy to obtain. Usually, the interesting results in electroporation research start where the electrical parameters of available electroporators are ending/out of range. For the specific application, the choice, in principle, is easier as the pulse parameters have been optimized before; the load is well characterized and so the range of parameters is narrowed. There are quite a number of laboratory electroporators available on the market but only a few are available for use in treating patients.

Acknowledgments

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