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Review

Techniques of signal generation required for electropermeabilization. Survey of electropermeabilization devices

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Abstract

Electropermeabilization is a phenomenon that transiently increases permeability of the cell plasma membrane. In the state of high permeability, the plasma membrane allows ions, small and large molecules to be introduced into the cytoplasm, although the cell plasma membrane represents a considerable barrier for them in its normal state. Besides introduction of various substances to cell cytoplasm, permeabilized cell membrane allows cell fusion or insertion of proteins to the cell membrane. Efficiency of all these applications strongly depends on parameters of electric pulses that are delivered to the treated object using specially developed electrodes and electronic devices—electroporators. In this paper we present and compare most commonly used techniques of signal generation required for electropermeabilization. In addition, we present an overview of commercially available electroporators and electroporation systems that were described in accessible literature.

Keywords: Electroporation; Electropermeabilization; Instrumentation; Electrochemotherapy; Gene transfection

1. Introduction

The use of high voltage electric pulse technology, electropermeabilization, in cell biology, biotechnology and medicine has attracted significant interest ever since first reports were published several decades ago [1–3]. Electropermeabilization is a transient phenomenon that increases permeability of the cell plasma membrane. In the state of high permeability, the plasma membrane allows ions, small and large molecules to be introduced into the cytoplasm, although the cell plasma membrane in its normal state represents a considerable barrier for them. Besides introduction of different substances to the cytoplasm, the permeabilized cell membrane allows cell fusion or insertion of proteins into cell membrane (Fig. 1) [4–7]. Efficacy of electropermeabilization and its applications strongly depends on many parameters that can be divided into parameters of the electric field (i.e. pulse amplitude, pulse duration, pulse repetition frequency, number of pulses and pulse shape) [8–13], and parameters that define the state of cells, their surroundings and cell geometry (i.e. temperature, osmotic pressure, cell size and shape, etc.) [7,14]. With properly chosen values of the electric field parameters, the process of electropermeabilization is reversible and cells return into their normal physiological state. If these parameters exceed certain values (e.g. amplitude of pulses is too high or duration of pulses is too long), cells are irreversibly permeabilized and lose their viability (Fig. 1) [5–7].

Permeabilization of cell plasma membrane is achieved by exposure of the cell to a short but intense electric field. The basic quantity underlying this process is presumably the induced transmembrane potential difference, which is in the first approximation proportional to the product of the applied electric field strength E and cell radius R [7,16]. Furthermore, it has been shown that electric field controls the permeabilization of cell membrane in two ways. (1) Electric field initiates permeabilization of cell membrane in the regions where transmembrane potential difference exceeds the threshold value (between 200 and 300 mV) [7,9]. (2) Electric field strength defines the size of permeabilized area of cell membrane [7,9,11]. This means that permeabilization of cell membrane will occur only if the applied electric field is larger than the threshold value. Since the induced transmembrane potential difference is also proportional to the cell radius, it is

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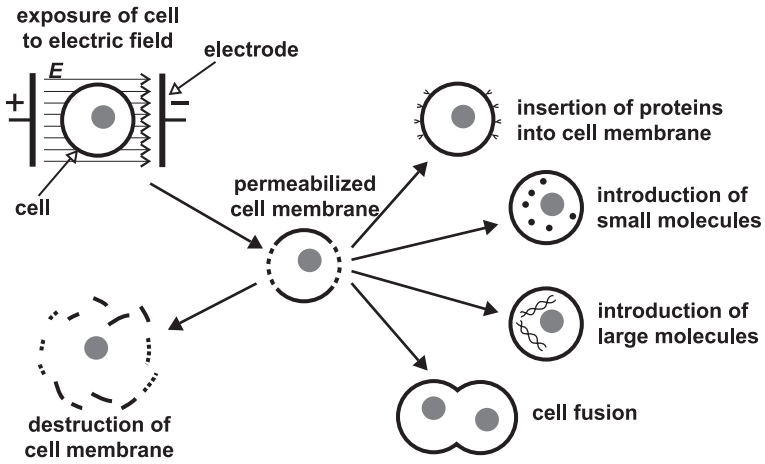

Fig. 1. Exposure of a cell to an electric field may result either in permeabilization of cell membrane or its destruction. In this process the electric field parameters play a major role. If these parameters are within certain range, the permeabilization is reversible, therefore it can be used in applications such as introduction of small or large molecules into the cytoplasm, insertion of proteins into cell membrane or cell fusion.

evident that threshold value of electric field varies with cell size. This means that large cells are more sensitive to lower electric field strengths than small cells [7,9]. Moreover, it has been shown that induced transmembrane potential difference also depends on cell density, arrangement and cell position [17–19]. Considering this, it is very difficult to generalize the electric field parameters for different experimental conditions (i.e. single cell permeabilization, in vitro, in vivo, etc.), or for different cell types (i.e. animal, plant, fungi, prokaryotic). In addition, different applications require different time variation of electric fields (i.e. exponentially decaying, square wave, etc.) and different exposure times.

It is not an aim of this paper to focus on further description of electric field parameters that are required in different applications of electropermeabilization. Instead, we would like to present and compare advantages and drawbacks of the existing and most commonly used concepts of electric signal generation and available devices that fulfill electrical requirements of applications such as: electrochemotherapy, electrotransfection, insertion of proteins into cell membrane, cell fusion and transdermal drug delivery [5–7,15,20–23].

2. Techniques of signal generation required for electropermeabilization

Effectiveness of electropermeabilization in either in vitro, in vivo or clinical environment depends on the distribution of electric field inside the treated sample [24–26]. To achieve this, we have to use an appropriate set of electrodes (e.g. needle, parallel plates, cuvettes, etc.) and an electropermeabilization device—electroporator that generates required voltage or current signals. Although both parts of the mentioned equipment are equally important for effectiveness of electropermeabilization, electroporator has substantially more important role since it has to be able to deliver the required signal to its output loaded by impedance of sample between electrodes.

Probably the major problem that every engineer faces during the design of electroporator is characterization of the load, which in principle has resistive and capacitive component. The value of each component is defined by geometry and material of electrodes and by electrical and chemical properties of the treated sample. In in vitro conditions these parameters that influence on impedance of load can be well controlled since size and geometry of sample is known especially if cuvettes are used, furthermore by using specially prepared cell mediums electrical and chemical properties are defined or can be measured [27–30]. On the other hand, in in vivo or clinical conditions, size and geometry can still be controlled to a certain extent but electrical and chemical properties can only be estimated. But what is practically impossible to predict during the development of the device are changes in the electrical and chemical properties of the sample due to exposure to high-voltage electric pulses. Besides electropermeabilization of cell membranes which increases electrical conductivity of the sample [31–33,38,39], electric pulses also cause at least two known side effects: heating and electrolytic contamination of the sample [10,34–37]. Furthermore, there are several other side effects that evolve from interactions between electrodes and treated sample, but we will not explain their influence on electrical and chemical properties of the sample because this is beyond the scope of this paper.

When most of the electrical parameters that electroporator should provide are determined, engineer has to choose the type of electroporator he is going to design. In principle, electroporators can be divided in several groups depending on the biological applications, but from the electrical point of view only two types of electroporators exist: devices with voltage output (output is voltage signal $U(t)$) and devices with current output (output is current signal $I(t)$). Both types of devices have their advantages and disadvantages, but one

point definitely speaks in favor of devices with voltage output. For example, if we perform in vitro experiments with stainless steel parallel plate electrodes with plate sides substantially larger than the distance between them, the electric field strength E that is applied to the sample can be approximated by the voltage-to-distance ratio U/d, where d is the electrode distance and U the amplitude of applied signal obtained from an electroporator with voltage output. On the other hand, if an electroporator with current output is used, the same approximation could be used only if additional measurement of voltage difference between electrodes is performed or if the impedance Z of the sample is known, measured or approximated and voltage difference between electrodes is estimated using Ohm's law $U=IZ$. This example shows that if an electroporator with voltage output is used, estimation of applied electric field strength can be made without additional measurements or knowledge of samples passive electrical properties.

Since electroporators with voltage output are much more widespread than the electroporators with current output, we will concentrate on most commonly used techniques to generate voltage signals required for electropermeabilization.

2.1. Discharge of a capacitor

This is the oldest technique used to generate signals for electropermeabilization primarily in in vitro environment. The device consists of: high voltage power supply, capacitor, switch, and optionally resistance (Fig. 2). The device operates in two phases, charge and discharge, and generates exponentially decaying pulses. During the charge phase, the switch (S) is in the position 1 and variable high voltage power supply (V) charges the capacitor (C) to the preset voltage. In the discharge phase, the switch is in the position 2, and the capacitor discharges through the load connected to the output. Time constant of discharge τ can be approximated by product $Z_L C$, where C is the capacitance of capacitor and Z_L is the absolute value of the load impedance. But most commercially available devices have built-in resistances that are connected in parallel to the load. Their main purpose is to define exactly the time

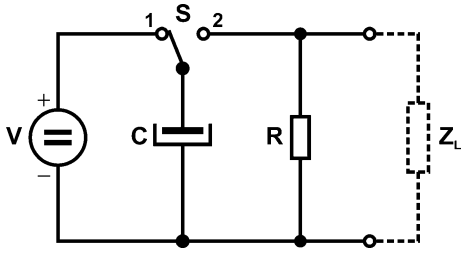

Fig. 2. Discharge of a capacitor (generator of exponentially decaying pulses). The basic setup comprises: variable high-voltage power supply (V), capacitor (C), switch (S), and optionally resistance (R). The device operates in two phases: charge (switch is in position 1 and capacitor charges to the preset voltage) and discharge (switch is in position 2 capacitor discharges through the load connected to the electrodes).

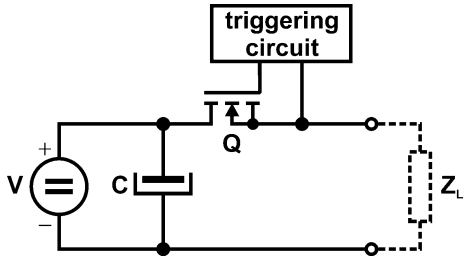

Fig. 3. Switching high voltage power supply with power transistors (generator of square wave pulses). The device consists of power supply part and pulse generator. The variable high-voltage power supply (V) continuously charges the capacitor (C) that stores energy required during the pulse. To deliver the pulse to the electrodes, the triggering circuit generates low-voltage pulse, usually around 10 V, that opens transistor (Q) (e.g. fast power MOSFET or IGBT) for the duration of the low-voltage pulse.

constant of discharge, since the impedance of load (e.g. cell suspension) varies [38–40]. If additional resistors are connected in parallel to the output the time constant of discharge is defined by: $(R||Z_L)\ C$, where R is resistance of the internal resistor. If 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.

The presented concept is very simple and the generated pulse could be used even for gene transfection since it includes the high voltage part for permeabilization and low voltage electrophoretic part [54]. Although the transition from high voltage to low voltage is smooth, the respective lengths of each part is ill-defined. Definition of electric field parameters is probably the major drawback of the presented technique. Moreover, repetition frequency of signal delivery is low due to a long charge phase, and the flexibility of electric field parameters is in general poor. Besides this, the presented technique usually requires additional circuits to prevent sparking that might be caused during the change of switch position.

2.2. Square wave generators

For better control of electric field parameters, square wave pulse generator has been introduced. The device still comprises a variable high voltage power supply (V) and a capacitor (C) for energy storage, yet the switch is replaced with a fast power MOSFET (metal oxide silicon field effect transistor) or IGBT (insulated gate bipolar transistor) (Q) and a triggering circuit (Fig. 3). In principle, such a device can continuously deliver square wave pulses to the output, provided that the high voltage power supply is able to recharge the capacitor during the delay between two consecutive pulses. The output amplitude of pulses is defined by amplitude of variable power supply, while pulse duration, pulse repetition frequency and possibly number of pulses are programmed by a computer that also comprises triggering circuit.

Despite improved control over the electric field parameters, this technique still has drawbacks that limit flexibility and accuracy of pulse parameters available to the user. The main problem lies in limited power capabilities of high voltage power supply. The charging current of capacitor that is delivered from power supply is usually much smaller than the discharging current that flows through the load during the pulse. Since more charge is taken from the capacitor than delivered, the voltage on capacitor decreases, which results in a decrease of pulse amplitude. The decrease of voltage can be limited by increasing capacitance of the capacitor, or it can be totally eliminated by using power supply that meets power requirements of the load. Because the first solution to the problem is more common, the accuracy of pulse amplitude of delivered pulses is within the range of few percent of the maximum value. In addition, limited power supply also influences the limitation of pulse duration and pulse repetition frequency. If consecutive pulses are generated, it is usually required that each pulse has the same amplitude as the first one that was generated. Due to the decrease of voltage on the capacitor during the pulse, next pulse can be delivered only after the capacitor is recharged to the preset voltage.

Despite these drawbacks, square wave pulse generators are still very often used to generate pulses especially in combination with pulse transformers (Fig. 4). This technique requires a square wave generator that generates low voltage pulses, while pulse transformer (T) outputs a high voltage pulse due to translation function that is defined by its properties. Furthermore, this configuration provides great safety margin because by using pulse transformer, the output floats and pulse transformer can be built to saturate if the pulse length exceeds the maximum pulse length [41,42].

Improved safety reduces the flexibility of pulse parameters, and while amplitude of pulses can be as high as 3 kV, pulse duration and pulse repetition frequency are limited by the characteristics of the pulse transformer. Despite the safety feature of the pulse transformer, it has to be stressed that development of such a transformer is

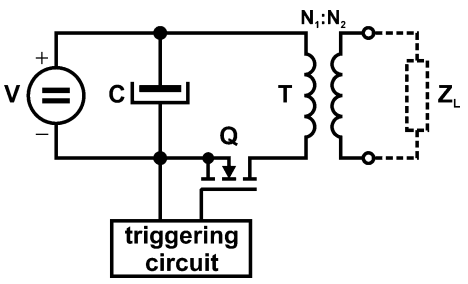

Fig. 4. Square wave pulse generator with pulse transformer. Similarly to the previous technique (see Fig. 3) the device comprises power supply and pulse generator, but between the load Z_L and pulse generator there is also pulse transformer (T) that additionally increases the amplitude of pulses.

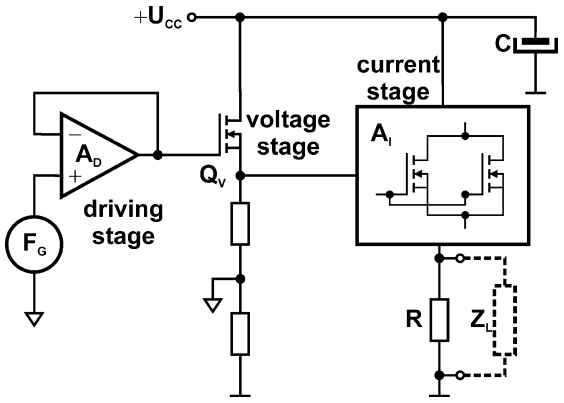

Fig. 5. Simplified circuit of an analogue generator of arbitrary signal. The signal generated by function generator F_G is delivered through the unity gain amplifier to the voltage stage, where the amplitude of signal is increased. The amplitude of signal delivered from driving stage (i.e. function generator and unity gain amplifier) defines the output amplitude of voltage stage. The signal then enters the current stage, which ensures the power required by the load Z_L.

complex due to nonlinear relationship between magnetic field density (B) and magnetic field strength (H) in the core of transformer. Beside this, additional output circuits are usually necessary to demagnetize the transformer after the end of the pulse. With no additional circuit at the output, demagnetization is carried out through the load, and consequently the shape of the signal is distorted (i.e. quasi bipolar pulses are produced).

2.3. Analogue generator of unipolar arbitrary signals

Although square wave and exponentially decaying pulses were and probably still are most frequently used signals for electropermeabilization, in some experiments pulses of different shape (e.g. trapezoidal pulses with possibility of control of rise and fall time or square wave pulses modulated with high-frequency sinusoidal signals) have been used [13,43].

For generation of arbitrary unipolar signals, technique requires at least two amplification stages (voltage and current) and appropriate driving stage (Fig. 5) [44]. The driving stage consists of a signal generator (F_G), which is usually a computer with a digital-to-analog converter, and a unity-gain amplifier (A_D) that meets power and impedance requirements at the input of a voltage stage. The voltage stage in the presented case is composed of a MOSFET (Q_V) and a resistor network connected to the source of the transistor. The signal delivered to the input of the voltage stage opens the transistor according to the transfer function, thus the output voltage changes (e.g. input of 4 V results in 200 V at the output). The major drawback of such voltage stage is that the ground of voltage stage must be electrically isolated from the ground of the driving stage. The signal is then delivered to the current stage, which is a classical source follower made of power MOSFETs connected in

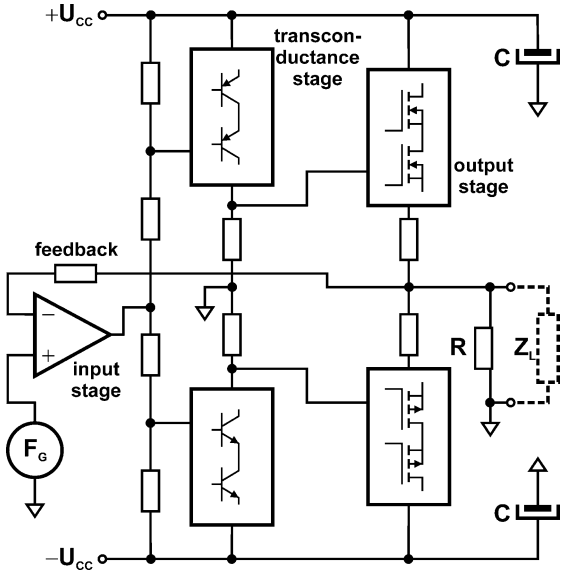

Fig. 6. Simplified circuit of an analogue generator of bipolar arbitrary signal. The signal generated by an arbitrary signal source (F_G) is delivered to the input stage where the signal is subtracted from attenuated output signal delivered through the feedback network. The differential signal is delivered to the inputs of two transconductance stages that increase voltage of signal (upper stage for positive signal and lower stage for negative signal). The two signals from each transconductance stage are then delivered to two output stages, where signals are recombined and amplified to meet power requirements required by load Z_L [47].

parallel. This last stage meets the power requirements determined by the impedance of load (Z_L) between the electrodes [45].

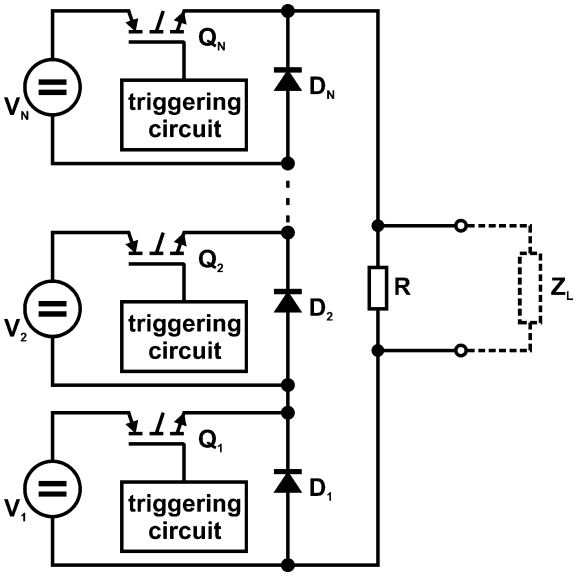

Fig. 7. Simplified circuit of modular high-voltage source. Operation of the device is based on a principle of digital-to-analogue converter, thus the device comprises several (N) individually controlled electrically isolated DC voltage modules, where the amplitude of the particular voltage source V_N is twice as high as in the preceding module. With an appropriate control of output transistors Q_1–Q_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 [48].

This design allows wide flexibility of all electrical parameters, yet some drawbacks still exist. The driving stage is much more complex than in previously described techniques, and besides this, it must have electrically isolated power supplies. With this design it is possible to generate signals with maximal amplitudes that are approximately 20 to 30 V lower than supply voltage ($+U_{CC}$). Probably the major problem remains general limitation of output voltage and current due to limitations of semiconductor technology (SOA-safe operation area of transistors).

2.4. Analogue generator of bipolar arbitrary signals

Until now we presented techniques that are only able to deliver unipolar signals. But some researchers in the field of electropermeabilization tend to utilize bipolar signals [9,10,46]. Today probably one of the best techniques that have been evaluated is a class AB bipolar amplifier, in other words the closed-loop push–pull amplifier (Fig. 6) [47].

The signal generated by an arbitrary signal source (F_G) is delivered to the input stage where the signal is subtracted from appropriately reduced output signal delivered through the feedback network. The difference of the two signals is delivered to the input of a bipolar voltage amplifier that comprises two transconductance stages, one for the positive and one for the negative period of the signal. Each amplifying stage is composed of two bipolar transistors (PNP-

Table 1
Comparison of presented techniques of signal generation for electropermeabilization

Technique	Advantages	Disadvantages
Discharge of capacitor	–simple and inexpensive construction	–poor flexibility of parameters
Square wave generator (power transistors)	–simple construction –better control of pulse parameters	–limitation of output parameters due to semiconductor technology
Square wave generator (pulse transformer)	–very safe (possibility to use in clinical environment) –very high pulse amplitudes	–limitations of pulse duration and repetition frequency –complex design of pulse transformer
Analogue generator of unipolar arbitrary signals	–wide flexibility of pulse parameters –arbitrary signal shape	–limitation of output current and voltage due to semiconductor technology
Analogue generator of bipolar arbitrary signals	–genuine bipolar arbitrary signals –arbitrary signal shape	–limitation of bandwidth, output current and voltage due to semiconductor technology
Modular high voltage source	–high dynamics –high currents and voltages	–price

Table 2
List of commercially available electroporators with their parameters, biological applications and possible signal generation technique

Company/product	Output characteristics	Voltage range	Time constant (τ)/pulse length (T)	Charge time (t_c)/pulse repetition frequency (f)	Biological application	Possible signal generation technique
ADITUS MEDICAL http://www.aditusmedical.com						
CythorLab	Arbitrary	LV: 0 V–600 Vpp, HV: 0 V–3000 Vpp	LV: T=400 ms HV: T=5 ms	NA	*in vitro*, *in vivo*	NA
AMAXA Biosystems http://www.amaxa.com						
Nucleofector	NA	NA	NA	NA	*in vitro* transfection	NA
BIORAD http://www.biorad.com						
Micro Pulser	Exponential	200–3000 V	τ=1–4 ms	t_c=5 s	bacterial, yeast	Capacitor discharge
Gene Pulser Xcell	Exponential Square wave	10–3000 V	τ=0.5 ms–3.3 s T=0.05–10 ms	t_c=5 s f=0.1–10 Hz	all cell type, eukaryotic and prokaryotic cells	Capacitor discharge
BTX http://www.btxonline.com						
ECM 399	Exponential	LV: 2–500 V HV: 10–2500 V	LV: τ=157 ms HV: τ=5.4 ms	t_c<5 s	bacterial, yeast, mammalian	Capacitor discharge
ECM 630	Exponential	LV: 10–500 V HV: 50–2500 V	LV: τ=25 μs–5 s HV:τ=625 μs–78 ms	t_c<5 s	bacterial, yeast, mammalian, plant, *in vivo*	Capacitor discharge
ECM 830	Square wave	LV: 5–500 V HV: 30–3000 V	LV: T=10 μs–10 s HV: T=10–600 μs	f=0.1–10 Hz	bacterial, yeast, mammalian, plant, *in vivo*, *in ovo*	LV: square wave generator HV: pulse transformer
ECM 2001	Square wave Sinus (AC)	LV: 10–500 V HV: 10–3000 V 0 V–150 Vpp	LV: T=10 μs–99 ms HV: T=1–99 μs f_{AC}=1 MHz	NA NA	mammalian, plant, electrofusion	LV: square wave generator HV: pulse transformer AC: NA
HT 3000	Square wave	LV: 0–500 V HV: 0–3000 V	LV: T=10 ms–1 s HV: T=10–600 ms	f=0.1–10 Hz	*in vitro*	LV: square wave generator HV: pulse transformer
CLONAID http://www.clonaid.com						
RMX2010	Square wave	5–200 V	T=10 μs–990 ms	f=1–10 Hz	gene transfection	Square wave generator
CYTO PULSE SCIENCES http://www.cytopulse.com						
PA-2000	Square wave	5–1000 V	T=1 μs–2 ms	f<8 Hz	*in vitro*, *in vivo*, *ex vivo*	Square wave generator
PA-4000	Square wave	5–1100 V	T=1 μs–2 ms	f<8 Hz	*in vitro*, *in vivo*, *ex vivo*	Square wave generator
PA-101	Sinus (AC)	10–150 Vpp	f_{AC}=0.2–2 MHz		dielectrophoresis	AC: NA
EPPENDORF SCIENTIFIC http://www.eppendorf.com						
Electroporator 2510	Exponential	200–2500 V	τ=5 ms	t_c<8s	bacterial, yeast	Capacitor discharge
Multiporator:						
Eukaryotic module	Exponential	20–1200 V	τ=15–500 μs	t_c<30s	mammalian, plant, oocytes	Capacitor discharge
Bacterial module	Exponential	200–2500 V	τ=5 ms	t_c<30 s	bacterial, yeast	Capacitor discharge
Fusion module	Square wave sinus (AC)	0–300 V 2–20 Vpp	T=5–300 μs f_{AC}=2 MHz	f=1 Hz	mammalian, plant	Square wave generator AC: NA
EQUIBIO http://www.equibio.com						
Easyjec T Plus	Exponential	100–3500 V	τ=10 μs–7 s	NA	all cell types	Capacitor discharge
Easyjec T Optima	Exponential	20–2500 V	τ=1.5 ms–7 s	NA	all cell types	Capacitor discharge
Easyjec T Prima	Exponential	1800–2500 V	τ=5 ms	NA	bacterial	Capacitor discharge
GENETRONICS http://www.genetronics.com						
MEDPULSER	Square wave	NA	NA	NA	electrochemotherapy, clinical device	NA
IGEA http://www.igea.it						
Cliniporator	Square wave	LV: 20–200 V HV: 50–1000 V	LV: T=10 μs–20 ms HV: T=30–200 μs	f=1 Hz–10 kHz	electrochemotherapy, gene therapy, clinical device	unipolar arbitrary generator

Table 2 (*continued*)

Company/product	Output characteristics	Voltage range	Time constant (τ)/pulse length (T)	Charge time (t_c)/pulse repetition frequency (f)	Biological application	Possible signal generation technique
JOUAN						
Electropulsator PS10	Square wave	0–1000 V	T=5 μs–24 ms	f=1–10 Hz	bacterial, yeast, mammalian, plant	Square wave generator
Electropulsator PS15	Square wave	0–1500 V	T=5 μs–24 ms	f=1–10 Hz	bacterial, yeast, mammalian, plant	Square wave generator
PROTECH INTERNATIONAL http://www.protechinternational.com						
CUY-21	Square wave	LV: 0.1–199 V HV: 200–500 V	LV: T=0.1–999 ms HV: T=0.1–100 ms	f=0.1–10 Hz	*in vitro*, *in vivo*, *in ovo*, *in utero*	Square wave generator
LF101	Square wave	0–999 V	T=5–99 ms	f=0.1–10 Hz	mammalian, plant, electrofusion	Square wave generator
TRITECH RESEARCH http://www.tritechresearch.com						
Mammo Zapper	Exponential	NA	NA	t_c=15 s	mammalian	Capacitor discharge
Bacto Zapper	Exponential	<2000 V	τ<10 ms	t_c=5 s	Bacterial	Capacitor discharge
THERMO ELECTRON CORPORATION http://www.savec.com						
CelljecT Uno	Exponential	1800 or 2500 V	τ=5 ms	NA	bacterial, yeast	Capacitor discharge
CelljecT Duo	Exponential	20–2500 V	τ=1.5 ms–7 s	NA	all cell type, eukaryotic and prokaryotic cells	Capacitor discharge
CelljecT Pro	Exponential	20V–3500V	τ=10 μs–7 s	t_c<30 s	bacterial, yeast, mammalian, plant	Capacitor discharge

Signal generation techniques that are given for each device were anticipated according to the output characteristic. During our investigation we did not have access to the electrical schemas of the devices nor had we any of the listed device in our hands.
NA stands for not available.

type for positive and NPN-type for negative period) connected in cascade and a resistor network necessary for normal operation. At this point it has to be stressed that complementary transistors have to be used (i.e. NPN and PNP type which are close match) otherwise symmetry between positive and negative part of amplifier cannot be achieved. The two signals amplified in each transconductance stage are delivered to two output stages, again one for positive and one for negative period of signal. The output stages are composed of power MOSFETs, if possible complementary (N-type for positive and P-type for negative period), that are connected in cascade as source followers. These last two stages recombine two signals from voltage amplifier and meet the power requirements defined by the impedance of the load between electrodes [47].

Although, the design by itself has no problems and is given as an example in any electronic design book, the major problem originates in poor availability of semiconductor components (i.e. high voltage and high power complementary transistors) necessary to build each of the amplifying stage. Since those transistors exist only up to 250 V, undesired cascades that gradually reduce dynamics have to be used to generate signals required for electropermeabilization.

2.5. Modular high voltage source

Another possible improvement of a square wave generator is a modular high voltage source that consists of several (N) individually controlled and electrically isolated DC voltage modules (Fig. 7). Its operation is based on a principle of a digital-to-analog 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 source is constant and can participate in a generation of a common output pulse at any time. With an appropriate control of output transistors Q_1–Q_N that operate as switches and connect the modules in series, a total of 2^N different output voltage levels with the resolution of V_1 are obtained [48]. Although the design of each individual source is similar to the design of previously described square wave pulse generator, the individual source used in this concept has no problems with the shortage of power. For correct operation, each source (even the smallest one) must be able to produce and sustain the maximum possible current during the pulse generation. If this is not ensured, the pulse amplitude will decrease.

The presented modular topology has many advantages due to very high dynamics and high power that can be delivered to its output. Furthermore, with a supplemented single-phase transistor bridge on the output, bipolar pulses can be generated as well. Besides the electrode polarity change, the transistor bridge also increases the incorporated safety measures of the device in case of malfunction, which could result in a delivery of huge power to the output. Namely, for any given pulse amplitude at least three power transistor switches have to be turned ON (two for the selection of the pulse polarity and at least one for the selection of the desired output pulse amplitude). The modular solution and consequently the increased number of

Table 3
List of commercially available electrodes with their properties and biological applications suggested by manufacturer

Company/product	Number of electrodes	Electrode distance	Needle length (L)/electrode size (shape)/Volume (V)	Electrode material	Biological application
BIORAD http://www.biorad.com					
CUVETTES: Compatible with Micro pulser, Gene Pulser Xcell					
	2	1 mm	V=100 μl	Aluminum	in vitro
	2	2 mm	V=400 μl	Aluminum	in vitro
	2	4 mm	V=800 μl	Aluminum	in vitro
BIOSMITH http://www.biosmith.com					
CUVETTES: Compatible with electroporation devices from all major manufacturers					
72001	2	1 mm	V=100 μl	Aluminum	in vitro
72002	2	2 mm	V=400 μl	Aluminum	in vitro
72004	2	4 mm	V=800 μl	Aluminum	in vitro
BTX http://www.btxonline.com					
2-NEEDLE ARRAY: Compatible with: ECM 830, 630, 395, 399, 600, T820					
Model 531	2	10 mm	L=200 mm	Stainless steel	in vivo
Model 532	2	5 mm	L=200 mm	Stainless steel	in vivo
GENETRODES: Compatible with: ECM 630, 830, 2001, 600, T820					
Model 508	2	1–10 mm	L=5 mm	Gold plating	in vivo
Model 510	2	1–10 mm	L=10 mm	Gold plating	in vivo
Model 512	2	0–13 mm	L=5 mm (L-shaped)	Gold plating	in ovo
Model 514	2	0–13 mm	L=3 mm (L-shaped)	Gold plating	in ovo
Model 516	2	0–13 mm	L=1 mm (L-shaped)	Gold plating	in ovo
CALIPER: Compatible with: ECM 830, 600, 630, 2001, T820					
Model 384	2	1–130 mm	10 × 10 mm (square)	Stainless steel	in vivo
Model 384L	2	1–130 mm	20 × 20 mm (square)	Stainless steel	in vivo
TWEZERTRODES: Compatible with: ECM T820, 630, 830, 2001					
Model 520	2	1–20 mm	7 mm diameter (disk)	Stainless steel	in vivo
Model 522	2	1–20 mm	10 mm diameter (disk)	Stainless steel	in vivo
GENEPADDLES: Compatible with: ECM 830, 2001, 630, 600, T820					
Model 542	2	1–10 mm	3 × 5 mm (rectangle)	Gold plating	in vitro, in vivo
Model 543	2	1–10 mm	5 × 7 mm (rectangle)	Gold plating	in vitro, in vivo
PETRI PULSER: Compatible with: ECM 830, 630, 600, 399, 395, T820					
PP35-2P	13	2 mm	V=0.5–30 ml	Gold plating	in vitro
PETRI DISH ELECTRODES: Compatible with:ECM 830, 630, 2001, 600, T 820					
	24	2 mm	V=10–50 ml	Stainless steel	in vitro
BTX http://www.btxonline.com					
MICROSLIDE: Compatible with: ECM 630, 830, 395, 399, 2001, 600, T820					
Model 450	2	0.5 mm	V=20 μl	Stainless steel	in vitro, fusion
Model 450-1	2	1 mm	V=40 μl	Stainless steel	in vitro, fusion
Model 453	2	3.2 mm	V=0.7 ml	Stainless steel	in vitro, fusion
Model 453-10	2	10 mm	V=2.2 ml	Stainless steel	in vitro, fusion
FLAT ELECTRODE CHAMBER: Compatible with: ECM 630, 830, 2001, 600, T820					
Model 484	2	1 mm	V=0.5 ml	Stainless steel	in vitro, fusion
Model 482	2	2 mm	V=1 ml	Stainless steel	in vitro, fusion
MEANDER FUSION CHAMBER: Compatible with: ECM 630, 830, 2001, 200, 600, T820					
	2	0.2 mm	–	Silver	in vitro, fusion
Electroporation plates:					
Model HT-P96-2B/W	96	2 mm	V=150 μl	Gold plating	in vitro
Model HT-P96-4B/W	96	4 mm	V=300 μl	Gold plating	in vitro
Model HT-P384-2B/W	384	2 mm	V=700 μl	Gold plating	in vitro
MULTI-WELL COAXIAL ELECTRODES: Compatible with: ECM 630, 830, 2001, 600, T820					
Model 491-1	1	1.6 mm	V=0.3 ml (circular)	Gold plating	in vitro
Model 747	8	1.6 mm	V=0.3 ml (circular)	Gold plating	in vitro
Model 840	96	1.6 mm	V=0.3 ml (circular)	Gold plating	in vitro
Flatpack chambers:					
Model 485	2	1.83 mm	V=1.5 ml	Stainless steel	in vitro

Table 3 (*continued*)

Company/product	Number of electrodes	Electrode distance	Needle length (L)/electrode size (shape)/Volume (V)	Electrode material	Biological application
Model 486	2	0.56 mm	V=85 μl	Stainless steel	in vitro
Cuvettes:					
Model 610	2	1 mm	V=20–90 μl	Aluminum	in vitro
Model 620	2	2 mm	V=40–400 μl	Aluminum	in vitro
Model 640	2	4 mm	V=80–800 μl	Aluminum	in vitro
EPPENDORF http://www.eppendorf.com					
CUVETTES: Compatible with Multiporator, Electroporator 2510					
	2	1 mm	V=100 μl	Aluminum	in vitro
	2	2 mm	V=400 μl	Aluminum	in vitro
	2	4 mm	V=800 μl	Aluminum	in vitro
CYTOPULSE http://www.cytopulse.com					
COAXIAL ELECTRODES: Compatible with PA-101					
Model FE-C25/400	2	2.5 mm	V=350 ml	NA	in vitro, fusion
Model FE-C25/800	2	2.5 mm	V=750 ml	NA	in vitro, fusion
Model FE-C20/1000	2	2 mm	V=1000 ml	NA	in vitro, fusion
Tweezers:					
TE-5-10	2	Adjustable	5 × 10 mm (rectangular)	NA	in vivo
TE-5R	2	Adjustable	5 mm diameter (circular)	NA	in vivo
2-row needle array:					
NE-4-4	8	4 mm		NA	in vivo
NE-4-6	12	4 mm		NA	in vivo
NE-6-4	8	6 mm		NA	in vivo
NE-6-6	12	6 mm		NA	in vivo
Cuvettes:					
CUV-01	2	1 mm	V=100 μl	NA	ex vivo
CUV-02	2	2 mm	V=400 μl	NA	ex vivo
CUV-04	2	4 mm	V=800 μl	NA	ex vivo
Electrode array:					
96W-A	96 wells	5.5 mm	V=300 μl/well	NA	ex vivo
EUROGENTEC http://www.eurogentec.com					
CUVETTES: Compatible with most existing electroporation systems					
	2	1 mm	NA	Aluminum	in vitro
	2	2 mm	NA	Aluminum	in vitro
	2	4 mm		Aluminum	in vitro
ICHOR http://www.ichorms.com					
Trigrid					
	multiple	NA	NA	NA	in vivo
IGEA http://www.igea.it.					
TYPE I: Compatible with Cliniporator					
Plate electrodes	2	6–8 mm	10 × 30 mm (rectangular)	Stainless steel	clinical applications
TYPE II: compatible with cliniporator					
Needle rows	8	4 mm	L=20–30 mm	Stainless steel	clinical applications
TYPE III: Compatible with Cliniporator					
Hexagonal needle array	7	8 mm	L=20–30 mm	Stainless steel	clinical applications

NA stands for not available.

assembly parts (isolated DC modules, IGBT driver circuitry, etc.), on the other hand, increase the costs of the device, which is a subject of optimization during the design stage.

3. Discussion

Nowadays electropermeabilization is widely used in various biological, medical, and biotechnological applications such as electrochemotherapy, gene transfer, electroinsertion of proteins into cell plasma membrane, electrofusion of cells, transdermal drug delivery, water treatment and food preservation [5–7,15,20–23,55–57]. Efficiency of all these applications strongly depends on parameters of electric pulses, which are delivered to the treated object using specially developed electrodes and electronic devices—electroporators. Both parts of equipment play equally important role in process of electropermeabilization, but in this paper we

have focused exclusively on electroporators and advantages and disadvantages of techniques used for generating required signals (Table 1). At this point we did not discuss how each of the presented techniques can solve different problems like tissue burning, electrolytic contamination, etc., since this would require additional analysis of electrode designs and materials.

Besides reviewing known techniques of signal generation, we also investigated the world market of electroporators. A list of existing commercially available electroporators with their parameters, biological applications and possible signal generation technique are given in Table 2. Devices are grouped by manufacturer and each device is presented with the following parameters: output characteristics, voltage range, time constant (τ)/pulse length (T), and charge time (t_c)/pulse repetition frequency (f). The value of last two parameters depends on output characteristic if the device produces exponentially decaying pulses, time constant and charge time are given as parameters. On the other hand, if the device generates square wave pulses, pulse length and pulse repetition frequency are given as parameters. Since some of manufacturers also offer different electrodes for different applications we have also made a list that is given in Table 3. Electrodes are grouped by manufacturers and each electrode is presented with the following parameters: electrode type, electrode distance and biological applications.

We can see that it is practically impossible to compare the listed devices due to difference in their characteristics. Even if we compare devices with identical output characteristic (e.g. exponential, square wave, arbitrary) we see that either their voltage range or their time constant/pulse length vary in incomparable range. We believe that with each of the listed devices adequate experimental results can be achieved, yet some questions still remain. Do we need any special buffers for electropermeabilization of cells? How can we set the required parameters for electropermeabilization (i.e. user friendliness of the device)? Is the device modular or non-modular type (i.e. with addition of new module we extend working parameters)? From this we can conclude that manufacturers of the electroporators have to standardize electrical parameters of devices, which would also include list of required buffers that have to be used for efficient electropermeabilization. This has already been done by some manufacturers (Eppendorf, BioRad, BTX, etc.) who supply protocols and standardized buffers for different procedures.

Besides standardization of parameters of devices, manufacturers should also start offering a built-in module for current and voltage monitoring. It is very important that researcher has an immediate feedback about the electropermeabilizing signal that has been delivered to the electrodes. Monitoring of voltage and current can be performed by use of an oscilloscope, but this requires additional space for another electronic device in already overstuffed laboratory and also additional wiring for signal measurements. In addition, researcher must also be able to set the oscilloscope before the experiments, which requires additional training. Probably there are many more drawbacks (e.g. expensive high voltage probes and current probes) of using the oscilloscope that could be overcome by built in current and voltage monitors.

Although today we can find several new studies showing biological effects of nanosecond pulsed electric fields [51,52], we did not review the parameters and technologies used, since this has already been done by Mankowski et al. [49]. In this review, they have presented several short pulse generator technologies such as discharge of capacitor, pulse forming line (PFL), Marxx-generator, etc. Besides this they also offer a list of commercially available short pulse generators.

In conclusion we can say that even though manufacturers offer a brand variety of electroporators and electroporation systems, these devices still have specific limitations. This was probably the main reason why many researchers have developed their own custom-designed devices or systems. Since many of these custom-built devices are poorly described in the articles, we were unable to explore their parameters in details. What we offer instead is a list of articles describing the devices (see Refs. [43,44,47–57]).

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References

[1] K. Kinosita, T.Y. Tsong, Formation and resealing of pores of controlled sizes in human erythrocyte membrane, Nature 268 (1977) 438–441.
[2] J. Teissie, T.Y. Tsong, Electric field induced transient pores in phospolipid bilayer vesicles, Biochemistry 20 (1981) 1548–1554.
[3] E. Neumann, M.S. Ridder, Y. Wang, P.H. Hofschneider, Gene transfer into mouse lyoma cells by electroporation in high electric fields, EMBO J. 1 (1982) 841–845.
[4] E. Neumann, A.E. Sowers, C.A. Jordan, Electroporation and Electrofusion in Cell Biology, Plenum, New York, 1989.
[5] L.M. Mir, Therapeutic perspectives of in vivo cell electropermeabilization, Bioelectrochemistry 53 (2000) 1–10.
[6] E. Neumann, S. Kakorin, K. Toensing, Fundamentals of electroporative delivery of drugs and genes, Bioelectrochem. Bioenerg. 48 (1999) 3–16.
[7] J. Teissie, N. Eynard, B. Gabriel, M.P. Rols, Electropermeabilization of cell membranes, Adv. Drug Deliv. Rev. 35 (1999) 3–19.
[8] M.P. Rols, J. Teissie, Electropermeabilization of mammalian cells to macromolecules: control by pulse duration, Biophys. J. 75 (1998) 1415–1423.

[9] T. Kotnik, L.M. Mir, K. Flisar, M. Puc, D. Miklavčič, Cell membrane electropermeabilization by symmetrical bipolar rectangular pulses: Part I. Increased efficiency of permeabilization, Bioelectrochemistry 54 (2001) 83–90.
[10] T. Kotnik, L.M. Mir, D. Miklavčič, Cell membrane electropermeabilization by symmetrical bipolar rectangular pulses: Part II. Reduced electrolytic contamination, Bioelectrochemistry 54 (2001) 91–95.
[11] M. Puc, T. Kotnik, L.M. Mir, D. Miklavčič, Quantitative model of small molecules uptake after in vitro cell electropermeabilization, Bioelectrochemistry 60 (2003) 1–10.
[12] G. Pucihar, L.M. Mir, D. Miklavčič, The effect of pulse repetition frequency on the uptake into electropermeabilized cells in vitro with possible analysis and it application, Bioelectrochem. Bioenerg. 57 (2002) 167–172.
[13] T. Kotnik, G. Pucihar, M. Reberšek, D. Miklavčič, L.M. Mir, Role of pulse shape in cell membrane electropermeabilization, Biochim. Biophys. Acta 1614 (2003) 193–200.
[14] M. Golzio, M.P. Mora, C. Raynaund, C. Delteil, J. Teissie, M.P. Rols, Control by osmotic pressure of voltage-induced permeabilization and gene transfer in mammalian cells, Biophys. J. 74 (1998) 3015–3022.
[15] J. Teissie, Transfer of foreign receptors to living cell surfaces: the bioelectrochemical approach, Bioelectrochem. Bioenerg. 46 (1998) 115–120.
[16] T. Kotnik, F. Bobanović, D. Miklavčič, Sensitivity of transmembrane voltage induced by applied electric fields: a theoretical analysis, Bioelectrochem. Bioenerg. 43 (1997) 285–291.
[17] P.J. Canatella, J.F. Karr, J.A. Petros, M.R. Prausnitz, Quantitative study of electroporation-mediated molecular uptake and cell viability, Biophys. J. 80 (2001) 755–764.
[18] M. Pavlin, N. Pavšelj, D. Miklavčič, Dependence of induced transmembrane potential on cell density, arrangement, and cell position inside a cell system, IEEE Trans. Biomed. Eng. 49 (2002) 605–612.
[19] B. Valič, M. Golzio, M. Pavlin, A. Schatz, C. Faurie, B. Gabriel, J. Teissie, M.P. Rols, D. Miklavčič, Effect on electric field induced transmembrane potential on spheroidal cells: theory and experiments, Eur. Biophys. J. 32 (2003) 519–528.
[20] M.R. Prausnitz, V.G Bose, R. Langer, J.C. Weaver, Electroporation of mammalian skin: a mechanism to enhance transdermal drug delivery, P. Natl. Acad. Sci. U. S. A. 90 (1993) 10504–10508.
[21] M.R. Prausnitz, A practical assessment of transdermal drug delivery by skin electroporation, Adv. Drug Deliv. Rev. 35 (1999) 61–67.
[22] S.B. Dev, D.P. Rabussay, G. Widera, G.A. Hofmann, Medical applications of electroporation, IEEE Trans. Plasma Sci. 28 (2000) 206–223.
[23] J. Gehl, Electroporation: theory and methods, perspectives for drug delivery, gene therapy and research, Acta Physiol. Scand. 177 (2003) 437–447.
[24] D. Miklavčič, K. Beravs, D. Šemrov, M. Čemažar, G. Serša, The importance of electric field distribution for effective in vivo electroporation of tissue, Biophys. J. 74 (1998) 2152–2158.
[25] J. Gehl, T.H. Sorensen, K. Nielsen, P. Raskmark, S.L. Nielsen, T. Skovsgaard, L.M. Mir, In vivo electroporation of skeletal muscle: threshold, efficacy and relation to electric field distribution, Biochim. Biophys. Acta 1428 (1999) 233–240.
[26] G. Serša, M. Čemažar, D. Šemrov, D. Miklavčič, Changing electrode orientation improves the efficacy of electrochemotherapy of solid tumors in mice, Bioelectrochem. Bioenerg. 39 (1996) 61–66.
[27] M.P. Rols, J. Teissie, Ionic-strength modulation of electrically induced permeabilization and associated fusion of mammalian cells, Eur. J. Biochem. 179 (1989) 109–115.
[28] E. Neumann, Membrane electroporation and direct gene transfer, Bioelectrochem. Bioenerg. 28 (1992) 247–267.
[29] C.S. Djuzenova, U. Zimmermann, H. Frank, V.L. Sukhorukov, E. Richter, G. Fuhr, Effect of medium conductivity and composition on the uptake of propidium iodide into electropermeabilized myeloma cells, Biochim. Biophys. Acta 1284 (1996) 143–152.
[30] G. Pucihar, T. Kotnik, M. Kandušer, D. Miklavčič, The influence of medium conductivity on electropermeabilization and survival of cells in vivo, Bioelectrochemistry 54 (2001) 107–115.
[31] I.G. Abidor, A.I. Barbul, D.Z. Zhev, P. Doinov, I.N. Bandarina, E.M. Osipova, S.I. Sukharev, Electrical properties of cell pallet and cell fusion in centrifuge, Biochim. Biophys. Acta 152 (1994) 207–218.
[32] I.G. Abidor, L.-H. Li, S.W. Hui, Studies of cell pallets: II. Osmotic properties, electroporation and related phenomena: membrane interactions, Biophys. J. 67 (1994) 427–435.
[33] R.V. Davalos, B. Rubinsky, D.M. Otten, A feasibility study for electrical impedance tomography as a means to monitor tissue electroporation for molecular medicine, IEEE Trans. Biomed. Eng. 49 (2002) 400–403.
[34] U. Pliquett, E.A. Gift, J.C. Weaver, Determination of the electric field and anomalous heating caused by exponential pulses with aluminum electrodes in electroporation experiments, Bioelectrochem. Bioenerg. 39 (1996) 39–53.
[35] U. Friderich, N. Stachowicz, A. Simm, G. Fuhr, K. Lucas, U. Zimmermann, High efficiency electrotransfection with aluminum electrodes using microsecond controlled pulses, Bioelectrochem. Bioenerg. 47 (1998) 103–111.
[36] F. Loste, N. Eynard, J. Teissie, Direct monitoring of the field strength during electropulsation, Bioelectrochem. Bioenerg. 47 (1998) 119–127.
[37] T. Tomov, I. Tsoneva, Are the stainless steel electrodes inter? Bioelectrochemistry 51 (2000) 207–209.
[38] M. Pavlin, T. Slivnik, D. Miklavčič, Effective conductivity of cell suspensions, IEEE Trans. Biomed. Eng. 49 (2002) 77–80.
[39] M. Pavlin, D. Miklavčič, Effective conductivity of a suspension of permeabilized cells: a theoretical analysis, Biophys. J. 85 (2003) 719–729.
[40] R.D. Fomekong, U. Pliquett, F. Pliquett, Passive electrical properties or RBC suspensions: changes due to distribution of relaxation times in dependence on the cell volume fraction and medium conductivity, Bioelectrochem. Bioenerg. 47 (1998) 81–88.
[41] G.A. Hofmann, S.B. Dev, G.S. Nanda, Electrochemotherapy: transition from laboratory to clinic, IEEE Eng. Med. Biol. Mag. 15 (1996) 124–132.
[42] M. Okino, H. Tomie, H. Kanesada, M. Marumoto, K. Esato, H. Suzuki, Optimal electric conditions in electrical impulse chemotherapy, Jpn. J. Cancer Res. 83 (1992) 1095–1101.
[43] D.C. Chang, Cell poration and cell fusion using an oscillating electric field, Biophys. J. 56 (1989) 641–652.
[44] M. Puc, K. Flisar, S. Reberšek, D. Miklavčič, Electroporator for in vitro cell permeabilization, Radiol. Oncol. 35 (2001) 203–207.
[45] M. Puc, S. Reberšek, D. Miklavčič, Requirements for a clinical electrochemotherapy device—electroporator, Radiol. Oncol. 31 (1997) 368–373.
[46] E. Tekle, R.D. Astumian, P.B. Chock, Electroporation by using bipolar oscillating electric field: an improved method foe DNA transfection of NIH 3T3 cell, P. Natl. Acad. Sci. U. S. A. 88 (1991) 4230–4234.
[47] K. Flisar, M. Puc, T. Kotnik, D. Miklavčič, A system for in vitro cell membrane electropermeabilization with arbitrary pulse waveforms, IEEE Eng. Med. Biol. Mag. 22 (2003) 77–81.
[48] M. Petkovšek, J. Nastran, D. Vončina, P. Zajec, D. Miklavčič, G. Serša, High voltage pulse generation, Electron. Lett. 38 (2002) 680–682.
[49] J. Mankowski, M. Kristiansen, A review of short pulse generator technology, IEEE Trans. Plasma Sci. 28 (2000) 102–108.
[50] Y.C. Lin, C.M. Jen, M.Y. Huang, C.Y Wu, X.Z. Lin, Electroporation microchips for continuous gene transfection, Sens. Actuators, B, Chem. 79 (2001) 137–143.
[51] S.J. Beebe, P.M. Fox, L.J. Rec, K. Somers, R.H. Stark, K.H. Schoenbach, Nanosecond pulsed electric field (nsPEF) effects on cells and tissues: apoptosis induced and tumor growth inhibition, IEEE Trans. Plasma Sci. 30 (2002) 286–292.
[52] K.H. Schoenbach, S. Katsuki, R.H. Stark, E.S. Buescher, S.J. Beebe,

Bioelectrics—new applications for pulsed power technology, IEEE Trans. Plasma Sci. 30 (2002) 293–300.
[53] A.C. Durieux, R. Bonnefoy, C. Manissolle, D. Freyssenet, High-efficiency gene electrotransfer into skeletal muscle: description and physiological applicability of a new pulse generator, Biochem. Biophys. Res. Commun. 296 (2002) 443–450.
[54] S. Šatkauskas, M.F. Bureau, M. Puc, A. Mahfoudi, D. Scherman, D. Miklavčič, L.M. Mir, Mechanisms of in vivo DNA electrotransfer: respective contributions of cell electropermeabilization and DNA electrophoresis, Molec. Ther. 5 (2002) 133–140.
[55] K. Uemura, S. Isobe, Developing a new apparatus for inactivating *Escherichia coli* in saline water with high electric field AC, J. Food Eng. 53 (2002) 203–207.
[56] J. Teissie, N. Eynard, M.C. Vernhes, A. Benichou, V. Ganeva, B. Galutzov, P.A. Cabanes, Recent biotechnological developments of electropulsation. A prospective review, Bioelectrochemistry 55 (2002) 107–112.
[57] K. Uemura, S. Isobe, Developing a new apparatus for inactivating bacillus subtilis spore in orange juice with a high electric field AC under pressurized conditions, J. Food Eng. 56 (2003) 325–329.