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Expert View

Functional Requirements and Quality Assurance Necessary for Successful Incorporation of Electroporation-Based Therapies Into Clinical Practice

Eva Pirc
Laboratory of Biocybernetics,
Faculty of Electrical Engineering,
University of Ljubljana,
Tržaška cesta 25,
Ljubljana 1000, Slovenia
e-mail: eva.pirc@fe.uni-lj.si

Matej Reberšek
Laboratory of Biocybernetics,
Faculty of Electrical Engineering,
University of Ljubljana,
Tržaška cesta 25,
Ljubljana 1000, Slovenia
e-mail: matej.rebersek@fe.uni-lj.si

Damijan Miklavčič[1]
Professor
Laboratory of Biocybernetics,
Faculty of Electrical Engineering,
University of Ljubljana,
Tržaška cesta 25,
Ljubljana 1000, Slovenia
e-mail: damijan.miklavcic@fe.uni-lj.si

Electroporation-based therapies have a huge potential for implementation into clinical practice in socioeconomically disadvantaged populations. Currently, the price of electroporators and electrodes is relatively high, but custom low budget devices can be developed. In the paper, we describe three most established applications in medicine, with the focus on the basic mechanisms, which should be taken into account during the development process of a clinical electroporator. Also, typical pulse parameters used in each of the described applications are defined. In the second part of the paper, we describe technical functional requirements for a clinical electroporator and safety guidelines, with the focus on medical device standard. At the end of the paper, the focus moves to a more general problematic, such as quality assurance and the importance of measurement during the pulse delivery, which we firmly believe is necessary for successful electroporation.
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1 Introduction

Electroporation is a phenomenon in which cells that are exposed to an intense pulsed electric field increase permeability and conductivity of their membranes. Each biological cell is surrounded by a cell membrane. The cell membrane consists mainly of phospholipids, which in aqueous conditions, due to its properties, form a bilayer. The bilayer is a stable structure [1] and due to its nonpolar interior, almost impenetrable for polar molecules, dissolved in the aqueous electrolyte. The resting transmembrane potential is in a range of few tens of mV, due to the distribution of ions between the cell exterior and interior [2]. If an external electric field is applied to a biological cell or tissue, the local electric field in the cell and their surroundings is disturbed. The imposed transmembrane voltage superimposes to the cell resting potential. The electrical field "amplifies" in the cell membrane, as the electric potential difference is established across the membrane. The induced transmembrane voltage is conditioned by the orientation of the cell regarding the applied electric field and cell shape. Therefore, due to the pulse application, transport through the cell membrane can be affected, cells can be stimulated, and if the applied electric field is high enough, pulse application can also lead to electroporation of the cell membrane.

According to current understanding, the electroporation is based on the formation of aqueous pores in the lipid bilayer [3–5]. Pores enable the ionic and molecular transport over otherwise impermeable membranes during the pulse. Experimental observation of the pore formation was not successful with known techniques, but molecular dynamic (MD) simulation and statistical thermodynamics provide convincing corroboration of pore formation. Recently, it was also shown that electric pulse exposure causes chemical changes to the lipids, responsible for the transport after the pulse, and modulation of membrane proteins' function [3].

We distinguish between reversible and irreversible electroporation. In the case of reversible electroporation, the cell after the pulse application fully recovers. Thus, through the permeabilized cell membrane, proteins and small or large molecules can be delivered. Additionally, if two cells are close to each other, fusion can occur, due to electroporation. But when the damage of the cell membrane and to the cell is too excessive, the cell dies, presumably due to the loss of cell homeostasis. Electroporation is a platform technology with many applications in different fields [6–9]. It is well established in medicine, where electrochemotherapy (ECT) has been studied for more than two decades [10]. With time, new applications developed and now nonthermal tissue ablation by means of irreversible electroporation (NTIRE) [11], DNA

[1]Corresponding author.

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vaccination [12], and gene therapy [13,14] are studied in detail. In the field of bio- and food technology, electroporation is exploited for inactivation of microorganisms, extraction of biomolecules, fast drying of biomass, and even cooking [8,9].

This paper aims at presenting the electroporation and electroporation-based therapies in medicine, as a prospect solution for socioeconomically disadvantaged people and populations. Because portable, battery-powered, cost-efficient clinical electroporator is not yet available on the market, we also provide guidelines for future developers.

In the first part of the paper, we describe three most established applications in medicine, with the focus on the basic mechanisms, which should be taken into account during the development process of a clinical electroporator. Additionally, for each application, we specify the range of used pulse parameters. In the second part of the paper, functional requirements for each of the previously described applications, safety guidelines, where we focus on compliance with medical device standard and FDA requirements, are defined. In the end, the focus moves to quality assurance and measurements.

2 Electroporation-Based Medical Applications

All electroporation therapies could be used at low cost and in areas with limited resources. Electroporator can be transportable and battery-powered and in case of wide pulse parameter range, could be, in combination with custom made electrodes, used for different medical applications (described below). Currently, electrodes for ECT and IRE are for single-use and quite expensive; however, this cost could and should be reduced with an increase in the use. The electroporator user interface can be developed in a way to facilitate the use; nevertheless, the device operator should be familiar with at least basic mechanisms of electroporation, meaning training is required. Electroporation-based therapies are safe, with little or no side effects, reduce stays in the hospital, can facilitate or replace surgeries and at the same time improve the quality of patient's life's, can be performed in an outpatient basis, and are therefore a promising application for introduction to the clinical practice all around the world. In this section, three applications are described, with the focus on the used electrical pulse characteristics (Fig. 1).

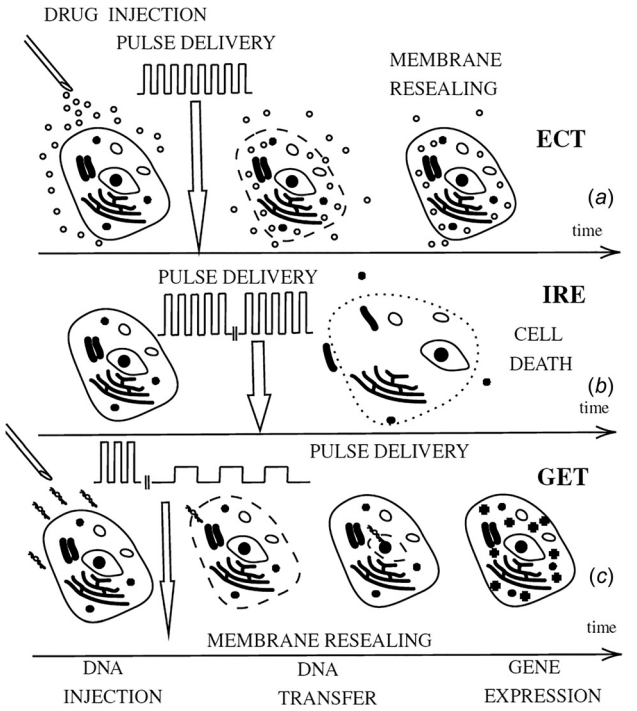

Fig. 1 Three most established electroporation-based medical applications: (*a*) ECT: after chemotherapeutic drug injection, electroporation pulses are delivered to the cell. Electroporation of cell membrane is triggered, which results in increased transport of the drug in the cell interior. (*b*) IRE: after the pulse delivery, the cell dies, due to the loss of homeostasis. (*c*) GET: the injected DNA migrates to the cell membrane, where it is due to electroporation and electrophoretic force, transferred into the cell interior. DNA then migrates toward the nucleus, where it is transported across the nuclear envelope. Afterward, the result of successful GET is gene expression, which can be used for gene therapy and DNA vaccination.

2.1 Electrochemotherapy. The leading electroporation-based therapy is ECT, an antitumor therapy [15–17], at which after injection of the chemotherapeutic drug, locally applied high voltage electric pulses trigger transient permeabilization of cells in a tissue. In the case of an adequate application, all tumor cells [18] get permeabilized, meaning the diffusion, of previously injected chemotherapeutic drug into the cells is thus facilitated. This results in higher cytotoxicity [19] of injected drug. Bleomycin is the most utilized drug for ECT and the intravenous infusion is the most common, while Cisplatin is injected intratumorally [20]. For the success of the application, drug concentration in the treated area should be sufficient and the whole tumor should be exposed to high enough electric field [21]. ECT can be easily repeated, in case of tumor progression or in cases where tumor does not completely respond to ECT after the first treatment. ECT is highly effective, with complete response rates between 60% and 70% and objective response rates about 80%, after a single treatment [10]. It is suitable for the treatment of cutaneous and subcutaneous tumors of different histotypes, both skin and non-skin cancers, as well as metastases. In the last few years, ECT has also been established in deep-seated tumors, including bone metastases, liver malignancies, and pancreatic and prostate cancers, and gastrointestinal tumors. Additionally, lung and brain tumors are currently studied as potential future targets [22]. ECT is a treatment of choice especially when tumors are located close to major blood vessels and consequently not manageable with surgery [23]. European Standard Operating Procedures of Electrochemotherapy (ESOPE) have been established in 2006 [24]; however, SOP only defines ECT for skin tumors smaller than 3 cm in diameter. Therefore, an update of SOP was published in 2018, which provides guidelines for the treatment of primary and metastatic tumors (also >3 cm) of the skin, based on broad experience across treatment centers and medical specialties [20]. National Institute for Health and Care Excellence (NICE), in the United Kingdom, has recognized ECT as an integral part of the multidisciplinary treatment for patients with skin metastases of nonskin origin and melanoma (NICE interventional procedure guidance IPG 446) [25]. For easier understanding of the ECT procedure, we suggest watching the video published in the Journal of Visualized Experiments [26].

A combination of ECT with immunotherapy, GET, radiotherapy, as well as calcium electroporation are promising novelties on the ECT field [22]. Calcium electroporation has been investigated in vitro, in vivo, and in early clinical trials, and it lends itself as safe inexpensive antitumor treatment [27]. A drastic increase of intracellular calcium concentration triggers necrotic cell death, due to acute energy depletion [28]. Calcium affects normal and malignant cells differently (malignant cells are more sensitive to calcium electroporation); it can be administered by other medical professionals (not only oncologists), it is nonmutagenic, and has a long durability [29]. Therefore, calcium ECT seems specifically advantageous for economically and socially disadvantaged countries. However, calcium electroporation is currently performed mainly for tumors localized to the skin and in combination with intratumoral injection, additional attention should be paid to the risk of necrosis. According to first results, calcium electroporation response rates are comparable to bleomycin ECT rates [30] and the same electroporation device can be used for both. In classical

ECT and calcium electroporation, eight square wave 100 μs long pulses with a repetition rate of 1 Hz or 5 kHz are most commonly used (To ensure electroporation, the electric field in tissue should be higher than 400 V/cm [31].). Pulse voltage amplitude is electrode (distance between the electrodes) and target tissue-dependent; in most cases, it is somewhere between 200 and 1000 V in case of skin electroporation and up to 3 kV for deep-seated tumors. Also, a 5 kHz pulse repetition rate is more common due to shorter duration of electroporation; the sensation of only one application of electric pulses (less pain), meaning muscle contraction is present after the pulse application and an electrode displacement due to muscle contraction during pulse delivery is therefore reduced. The SOP [20] in detail defines treatment choices, including pretreatment examinations, anesthesia, drug injection, electrode selection, and characteristic of electric pulses for treatment with a Cliniporator device from IGEA. If a device or electrodes from other manufacturers or custom-made electrodes are used, the electric pulse characteristic should be adjusted to comply with requirements in the SOP.

2.2 Irreversible Electroporation. IRE or NTIRE-nonthermal irreversible electroporation is a promising application for ablation of nontumor and tumor tissue. The cells primarily die due to membrane permeabilization and not due to the increase of tissue's temperature [32,33]. However, a local temperature increases around the electrodes and can be significant at higher amplitudes, also due to the high number of pulses delivered to a limited volume of tissue [34,35]. It was shown that IRE does not cause denaturation of proteins and is not affected by blood flow. Additionally, rapid activation of the immune system, no scarring, and the potential ability to treat tumors near large blood vessels were observed [11]. In case of irreversible electroporation of tumor tissue, the same as in ECT, all tumor tissues should be covered with a high enough electric field; however, for IRE, the electric field should be above the IRE threshold, which is around 700 V/cm [11,36] for approximately 100 pulses. Therefore, to cross the IRE threshold, applied pulse amplitudes go as high as 3000 V, meaning current amplitude values can reach up to 50 A [37] (similar values can also be reached at ECT of deep-seated tumors). The number of applied pulses at IRE is most often above 90 and the pulse duration is around 100 μs. Because the standard operating procedures are not yet defined for the IRE, the pulse parameters of delivered pulses are much more diverse. In addition, an individual treatment plan is required for each specific tumor and is crucial for a successful outcome. IRE is mainly used for the treatment of deep-seated tumors either during open surgery or percutaneously in the liver, pancreas, kidney, lung, and other organs [38]. Because electric fields applied in IRE can cause cardiac arrhythmias, synchronization of pulse delivery with the refractory period of the cardiac rhythm is necessary [39]. The additional issue at IRE is muscle contractions [40], which are associated with the high voltage amplitudes and a large number of pulses delivered at a low repetition rate to avoid excessive tissue heating. Therefore, general anesthesia and neuromuscular blocking agents are necessary to prevent muscle contraction [11]. Recently, in a series of studies, authors showed that by applying bursts of high-frequency bipolar pulses also termed as H-FIRE (High-Frequency IrReversible Electroporation) pulses, muscle contractions can be avoided, without compromising the nonthermal mechanism of cell death [41,42]. Every single monopolar pulse is replaced by a burst of few microsecond long bipolar pulses, with a repetition rate in ranges of few hundred kHz, while the repetition rate of the burst stays the same as pulse repetition rate at IRE. Additionally, in the case of H-FIRE, even the electric field distribution is more homogeneous. Also, the transmembrane transfer of molecules may be achieved with bursts of short few microsecond long pulses; however, H-FIRE pulses need considerably larger voltage amplitudes for cell disruption in comparison to longer monopolar pulses [43]. Recent reports offer the possibility to use H-FIRE pulses in electrochemotherapy, but again, at the expense of higher electric fields than in classical ECT [44].

Tissue ablation with IRE would enable the ablation of previously unresectable tumors. Additional, IRE is in comparison to other treatment modalities that are easy to perform, favorable, because the procedure time is short, and it was also shown, IRE has a low risk of bleeding and perforation [45].

2.3 Gene Therapy and DNA Vaccination. Gene electrotransfection is a promising non-viral gene delivery method [13,46], used for treatment of cancer and other diseases [47,48], regulation of protein levels to enhance or reduce function, or the amelioration of symptoms of iatrogenic or natural disease, DNA vaccination [49,50], and genetic modification of organisms [51,52]. Plasmids or oligonucleotides may also be delivered to explore promoter or gene function [53]. Facilitation of gene expression in vivo by electroporation of plasmid DNA has implications for both vaccine and gene therapy applications [12]. Electroporation promotes antigen, oligonucleotides, and immunomodulatory molecule delivery into tumor tissue, which then stimulate the immune system or act on immunosuppressor genes [54]. Plasmid DNA should be injected before electroporation and should be always delivered to the tissue placed between the electrodes. After or during the electroporation, the cell membrane interacts with injected plasmid and forms a DNA-membrane complex, which is then transported through the cytoplasm to the nucleus. The electroporation has to be reversible because, after the pulse delivery, the cells have to express transferred genes [55]. Great variety of pulses with different pulse parameters are used in this field, both high-voltage short-duration pulses (few hundred μs long pulses and local electric fields around 400 V/cm (HV)) or low-voltage long-duration pulses (from few to hundreds ms long pulses and local electric fields of few tens V/cm (LV)) or even the combination of both. In some studies, it was suggested that HV pulses are crucial for permeabilization of the cell membrane and pore formation, while LV pulses electrophoretically drag negatively charged DNA into the cell [56]. In other studies, the authors proposed the change of electric field direction or orientation of the electrodes during the pulse delivery; thus, the area of DNA entry is enlarged (because DNA transfection occurs only in the part of the membrane facing the cathode) [57]. Additionally, nanosecond pulses with a duration from few to hundreds of nanoseconds and electric field densities up to several tens of kV/cm are currently studied, because it is believed that they affect internal cell membranes and may contribute to increased delivery of plasmid to the nucleus [58]. Overall, it was proven that longer electric pulses give higher transfection efficiency, but they reduce cell viability and shorter pulses give lower transfection efficiency and preserve viability.

In the field of DNA vaccination by electroporation, mainly intramuscular (IM) and intradermal (ID) delivery are studied. The muscle is an attractive tissue for nucleic acid vaccination in a clinical setting due to the accessibility and abundance of the target tissue [59]. Skeletal muscles promote strong humoral and cellular immune responses; however, muscle fibers are quite ineffective in plasmid capture. Therefore, for a successful application, a large volume of plasmid should be injected. The expression of plasmid DNA in the muscles can be prolonged and increased by electroporation. It was reported that by electroporation, the antigen delivery increase up to a 1000 fold over naked DNA delivery alone can be reached [50]. Currently, the development of delivery systems and research in the field of DNA vaccination by electroporation are at full swing [60]. Clinical studies of electroporation in the muscle showed the procedure is well tolerated by patients.

HIV, Human papillomavirus (HPV), Hepatitis C, and cancer DNA vaccination by electroporation are currently investigated [14]. For cancer gene therapy, viral delivery methods such as immune modulators, cell cycle regulators, suicide genes, anti-angiogenic genes, and genes encoding toxins are used and

electroporation mediated delivery is studied. It was shown that the most successful cancer therapeutic genes, delivered by electroporation, which results in significant tumor regression are IL-12 and IFN-α [48,53]. However, studies [61,62] also determined that optimization of electroporation pulse characteristics have a major impact on the success of the application; therefore, further research is necessary. Because vaccination is generally regarded to be one of the most cost-effective interventions in public health, we believe DNA vaccination with electroporation is the most promising application for implementation into economically disadvantaged populations. DNA is more heat stable, easier to produce and store than current vaccines [63]. The transport from the manufacturing to injection into the patient is easier and therefore cheaper, because DNA is more heat stable, and easier to produce and store than current vaccines.

3 Medical Devices for Electroporation

As presented in Sec. 2, different applications require different pulse parameters, such as voltage amplitude, pulse width, pulse repetition rates, and a number of pulses or even bursts of pulses [64,65]. Therefore, specific pulse generators, i.e., electroporators, have to be designed and developed for each application. When designing an electroporation device, one should always keep in mind that biological sample/tissue as a load has resistive–capacitive nature and varies from sample to sample. Additionally, also the impedance of a biological load decreases during the pulse delivery, due to electroporation [66,67].

A considerable number of electroporation devices can be found on the market, some designed for specific applications and some for multifunctional laboratory use. Several reviews have been published, in which pulse characteristics of commercially available electroporators are described [68–70]. But for the incorporation of an electroporator into the clinical practice, an electroporator has to follow the requirements appointed by local medical regulations and meet medical device standards. In Europe, it has to comply with a Medical Device Regulation 2017/745 and in the United States, the device should be approved by the FDA (Food and Drug Administration). The process of incorporation of a medical device in clinical practice in developed countries is thus well established. After the development process and collection of considerable scientific evidence for devices' safety and effectiveness, the first-in-human study is performed, followed by the evaluation of the device in clinical trials, culminating in regulatory approval for use and the adoption of the device [71]. The European CE Mark adoption process requires demonstration of safety, quality, and efficacy and nongovernmental notified bodies regulate the approval and postapproval. While in the United States, demonstration of safety and efficacy is required and regulated by a central governmental agency (FDA) [72]. All clinical trials, studies, or testing have to be authorized by the FDA. And even after a medical device is made available on the market for use, regulations have been established to ensure ongoing postmarket surveillance of device safety and effectiveness [73].

In Europe, currently there are only five certified clinical electroporators, the Cliniporator 2 and Cliniporator VITAE (IGEA S.p.A., Italy), available for ECT and GET, SENNEX (BionMed Technologies, Germany), that is only used for ECT, NanoKnife (AngioDynamics, USA), which may be used for soft-tissue ablation and ePORE (Mirai Medical, Ireland) that is compatible with the EndoVE, which enables a targeted treatment delivered within minutes in an outpatient endoscopy setting. In the United States, the FDA clearance was granted to NanoKnife (AngioDynamics, USA), first for the soft-tissue ablation and recently also for "Direct IRE Cancer Treatment" clinical study (DIRECT), for the treatment of stage III pancreatic cancer. Nevertheless, it has not received clearance for the therapy or treatment of any specific disease or condition. Unfortunately, none of those devices is actually appropriate for implementation in economically disadvantaged populations. The first issue is the cost, for example, a Cliniporator device is sold for 100,000 € (without VAT) and each electrode that is for single-use cost 1200 € and sometimes, more than one set of electrodes per treatment are used. Few cost-effectiveness studies of ECT have been done [74,75], but none of them have taken into account also the increase in the quality of life of patients after the ECT. Therefore, the use of ECT even in developed countries is sometimes hard to justify. Additionally, none of the existing clinical electroporators is battery-powered or easy to transport. Therefore, in the following part of the paper, we provide guidelines for future electroporator manufactures, based on our experiences. No relevant cost-effectiveness or economic studies have been published yet; therefore, it is hard to evaluate its potential for implementation in economically disadvantages population. However, in the case of the development of a low-cost electroporator for all presented applications it can have potential for implementation.

3.1 Functional Requirements. For successful electroporation and development of a quality and reliable electroporator, it is crucial to understand what needs to be achieved when applying electric pulses. Because different applications require different electric pulse characteristics, and because biological loads vary considerably, specific electroporators are designed for each application. However, it is possible to develop a device that could be used for all presented medical applications. Micro- and millisecond square wave pulses are usually generated by an HV power supply switching circuit, with fast power MOSFET or IGBT used as switches [76–79]. The simplest and most cost-efficient solution is a square wave generator concept shown in Fig. 2. The solution is similar to the capacitor discharge circuit; the voltage power supply V constantly charges the capacitor C. Pulse duration, pulse repetition rate, and a number of pulses are defined by the switching sequence, meaning the switch has to be driven by a precise and adequate driving/control system. The output pulse amplitude is defined by the set amplitude of the variable power supply. To minimize a voltage drop on the load, all the required energy must be generated and stored in the capacitor before each pulse delivery. Meaning large capacitor or capacitor banks are needed, especially in the case of IRE, where applied pulse voltage amplitudes are as high as 3 kV and at least 90 pulses are delivered. For the development of a nanosecond electroporator, more complex circuit designs are required such as a transmission line approach (Blumlein line) and diode opening switch and even faster switches are needed, mainly Radio Frequency MOSFET (RF-MOSFET) or Silicon Carbide MOSFET (SiC MOSFET) are used [80]. For the bipolar pulse generation, an H-bridge solution is the most established. The short rise time of high voltage pulses is provided by modular generator and short fall times can be achieved by short-circuiting of the load [81].

For specific treatments (i.e., applications, treatment locations), special application-optimized electrodes are used. SOP [20]

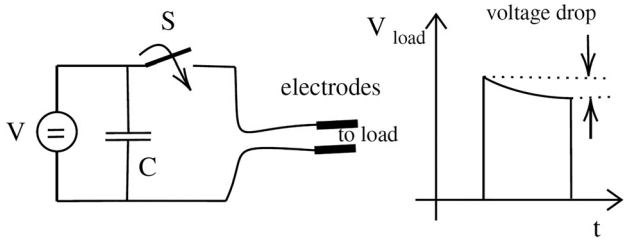

Fig. 2 Square wave generator concept. Switch S discharges capacitor C, through the load. The capacitor C is constantly charged, by a variable voltage source V. The amplitude of the generated pulse is equal to the set voltage of the source. The voltage drop during the pulse is capacitance, load impedance, and pulse duration dependent and should not exceed 10% of the set voltage V.

describes the appropriate use of different electrodes available for ECT in combination with the Cliniporator device. An electroporator should be compatible with different electrode types. However, the device should recognize the electrode type and adjust the output pulse parameters in accordance with the treatment modality. For example, if the distance between the electrodes is larger, then the pulse amplitude should be increased, to ensure the same electric field in the targeted tissue. An important part of the clinical electroporator, from the prospect of the use, is a user interface. Devices for the clinics should be as user-friendly as possible. To prevent misuse and mistakes, the output pulse characteristic should be preprogrammed for specific electrodes and treatment protocols. Meaning the user only selects, i.e., electrode type. However, in the case of deep-seated tumors, where anatomy is more complex, or due to the inability to cover the whole tumor due to its size, with standard electrodes, several single-needle electrodes are used [82]. Special treatment plans are designed for each therapy, to ensure the optimal configuration of the electrodes that adequately cover the tumor with high enough electric field. Meaning, a custom setting of the pulse parameter should be enabled in this case. Due to safety reasons, clinical electroporators are armed by the user, just before the pulse delivery. When capacitors are charged, the device is ready for pulse delivery; however, this active time is limited to a few seconds. If the device is not triggered and pulses are not delivered, it should self-discharge. In the case of IRE and ECT of deep-seated tumors, the pulse delivery should also be synchronized with cardiac rhythm. Both charge and delivery triggers should be made in the form of a pedal or enabling button on the handle, in a way not to obstruct the user. An electroporator should have a built-in measuring system, with an adequate bandwidth. The current and voltage should be constantly monitored to ensure the pulse delivery, with the sampling frequency that is equal or higher to twice the highest frequency contained in the measured signal. The real-time oscilloscope like display of the measurement is desirable but not mandatory. Nevertheless, at least the measured amplitude values of delivered voltage and current should be displayed as feedback and saved for post-treatment analysis if necessary. The evaluation of the delivered pulses is necessary, the device can self-evaluate the delivered pulses, or this can be done by the operator if adequate data are displayed.

3.2 Safety Guidelines. A clinical electroporator has to comply with the general standard for basic safety and essential performance of medical devices, EN/IEC 60601-1 [83]. The manufacturer should ensure that the risk is removed, or if not possible, the risk should be minimized. Therefore, manufacturers have to pay particular attention to choosing adequate voltage insulation, limit leakage currents in accordance with the safety class, limit the output voltage, current and energy and to ensure electromagnetic compatibility by following the standard EN/IEC 60601-1-2 EMC for medical devices.

Safety class of the electroporator is defined by its most risky application. We distinguish between invasive and noninvasive electroporation. If the device is made for more treatment modalities, it should be classified on the basis of the one that represents the highest risk. The same applies to the location of treatment; if the device can be used in surgery for deep-seated tumors, then the location with the highest risk has to be taken into account. Both Cliniporator EPS02 and Cliniporator VITAE have been in accordance with EN 60601-1 classified as Class I regarding the protection against electrical risks and class BF regarding the protection against electric shock. Cliniporator EPS02 in accordance with MDD 93/42 CEE classified as IIa, while Cliniporator VITAE is in class IIb. Additionally, also the following standards have to be considered: ISO 14971 for risk analysis, ISO 13485 for the quality management system, EN/IEC 60601-1-6 and ISO 62366 for usability, ISO 62304 and IEC 80002-1 for medical device software, and IEC 62311 in case of a battery power supply.

3.3 Quality Assurance. Currently, electroporation research and applications are developing fast and growing; therefore, the number of electroporators available on the market is increasing even faster than before. Unfortunately, the quality of some devices is questionable [70]. Some manufacturers intentionally conceal the output pulse parameters of their devices, claiming that it is their intellectual property. The critical point on the field of in vitro gene transfection, where preprogrammed electroporation procedures are most commonly used by researchers, without even knowing basic pulse parameters such as amplitude and pulse duration [70]. The concealed operation of electroporation devices hinders sharing, comparing, and reproduction of results; it limits and restricts researchers and further development of the new knowledge. In the electroporation field as well as in general biomedical research, the increase in failed efforts to confirm other group's published papers work is significant [84]. We believe this is at least in part due to poor quality assurance. Measurement of electroporation pulses is crucial to adequately determine and control the quality and the delivery of pulses. Even the International Medical Device Regulators Forum (IMDRF) gave the harmonization initiative and proposed mutual recognition encouraged by WHO. They engage an external consultant to conduct a more detailed study to examine overseas experience and practices of, and the scope of control on the use of, electroporation medical devices. We can conclude that standardization regarding quality is necessary; however, electroporation is a platform technology, and specific testing protocols for each application or field should be established eventually. Nevertheless, some basic technical specifications and tolerances can be applied to all electroporators. Currently, the manufactures often give ill-defined technical specification, defined pulse parameter ranges cannot be used with all biological loads and/or in all possible combinations. Meaning, for example, in case of low impedance load the number of pulses or pulse duration and the voltage range are limited. Therefore, before actual experiment, the researchers cannot know, if the device will be able to deliver what it promises. Thus, we address the future developers of electroporator medical as well as laboratory to follow the recommended guidelines.

Each device is designed for precisely defined maximal current and some limitation is necessary, for the protection of the device itself. Manufacturers should define the maximal current and the principle of current limitation. Some devices use a pre-pulse to evaluate the load and then adjust the pulse parameter range, in a way to disable the current to go over the limit. If pre-pulse is used, the amplitude and pulse duration should be as low as possible, but the pause between the pre-pulse and preset electroporation pulses should be long (All processes triggered by the pre-pulse must be extinguished before the delivery of actual electroporation pulses.). Pre-pulse parameters should be given in detail in technical specifications (e.g., the amplitude, pulse duration, and exact timing of the pre-pulse regarding the electroporation pulses). Other devices measure the current on the output and then stop the pulse delivery when the current value is too high. Such a measuring system should be fast enough to assure the disconnection within the pulse rise time and in this case, the user should be informed about the interruption of pulse delivery. The third solution is to build in an electroporator an additional impedance, which limits the output current. Meaning that the device will never be able to generate higher current than maximal current and will, therefore, reduce the applied voltage. Again, in this case, the user should be informed about the alternation. Therefore, we propose the following solution. The manufactures should define applications for which the device is made, and then provide the technical specification and tolerances in accordance with the typical load for those applications, e.g., voltage: 200–1000 V (for loads with impedance higher than 50 Ω and for pulse durations up to 1 ms ($N_{MAX} = 10$); 200–2000 V (for loads with impedance higher than 100 Ω and for pulse durations up to 100 μs ($N_{MAX} = 10$);); …, where N_{MAX} is the maximal number of pulses.

On the base of the literature review [66,85–87], our experiences, and current status of technology, we defined the tolerance of the voltage amplitude and pulse duration. Meaning the requirements could be easily fulfilled, with the available electronics and circuit solutions. Published permeabilization and cell survival curves [66,85–87] indicate that more than 15% deviation can result in significantly different electroporation outcome. However, in case of in vivo electroporation, an additional error can be present in electrode placement, treatment planning, muscle contractions (present during the pulse delivery) or other unexpected circumstances. Because the total tolerance is equal to the sum of all tolerances, which may exceed 15%, the developers of electroporation devices should at least follow the state-of-the-art tolerances. Meaning the requirements could be easily fulfilled, with the available electronics and circuit solutions. Therefore, the recommended voltage amplitude tolerance is 10%; thus, the applied voltage amplitude should not be lower than 90% or higher than 110% of preset voltage. Meaning also the voltage of the first to the last pulse should be in this range. The pulse duration should be defined as Full Width at Half Maximum (FWHM) and a deviation from the preset value should not be higher than 8% [85,86], as shown in Fig. 3. The proposed tolerances must be fulfilled, from maximal to minimal settings, which are: maximal pulse amplitude, maximal pulse duration, the maximal number of pulses, and minimal pulse repletion rate when the pulse is applied on a typical load for an application that it is made for. Meaning the list of applications should be defined and a typical load for each of those applications should be developed. Additionally, also electrodes should be standardized and tolerances of the distances between the electrodes, appropriate materials and in case of multiple use, also sterilization and maintenance should be defined. A clinical electroporator should constantly self-test the operation, and periodic evaluation and calibration of the device are mandatory. The tolerances defined in this section address the "classical" electroporation and are not applicable to the nanosecond electroporation, where pulse duration may be the dominant source of deviation.

3.4 Usage Guidelines. Each user as well as developer, of electroporation-based therapies, has to be familiar with basic electroporation mechanisms; therefore, education (e.g., www.ebtt.org) and training (e.g., ESSO course on ECT) are necessary. The application can be successful, only if the applied electric field covers the whole targeted area and if induced voltage is higher than the reversible or irreversible electroporation threshold voltage. It should always be taken into account that the tissue is not homogeneous, meaning the electric field, to which the biological load was exposed during the pulse delivery, cannot be evaluated simply by equation (E = U/d), which defines an electric field as the ratio of applied voltage amplitude and distance between the electrodes. This equation is only valid if the load between the electrodes is homogeneous and plate electrodes are used and the distance between the electrodes is small with respect to electrode dimensions. The operation of an electroporator has to be always monitored; only by adequate measuring, the quality of the pulse delivery can be assured. We need to measure because we need to know, if the pulses were successfully delivered and we need to know, what was delivered. If the current flows through a load, delivery was more or less successful, but we still do not know anything about the pulse characteristics of the delivered pulses. If an electroporator has a built-in measuring system, the user has to evaluate the quality of measurements displayed by the electroporator. Due to the poor regulation of the electroporation field and lack of standard, the quality of built-in measurement systems is sometimes questionable. Because of a large variety in the electrical characteristic of biological loads/tissues, the user has to be familiar with common errors in pulse delivery. When biological loads have low impedance, problems can occur because the pulse generators cannot deliver "what they promise," i.e., high voltage pulses due to too high currents. Or the amplitude of successive pulses can be lower with each successive pulse delivered, if the pulse repetition rate is in the higher half of device operation range, meaning that the electroporator's energy storage is not sufficient. In the worst case, the voltage amplitude can even be lower than electroporation threshold voltage [70]. Additionally, also each user should be familiar with the guidelines for reporting electroporation clinical studies [25].

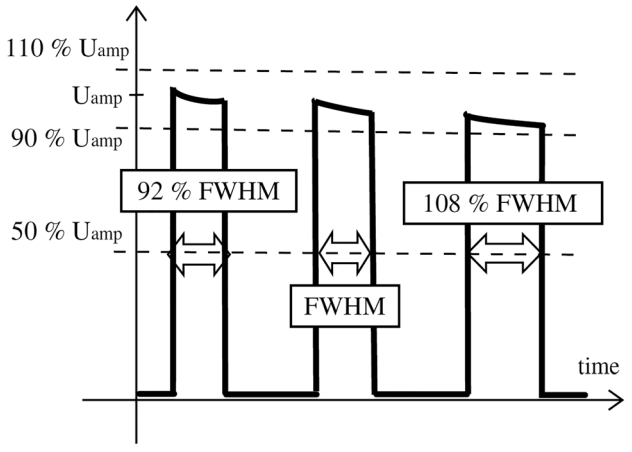

Fig. 3 The applied voltage amplitude should not be lower than 90% or higher than 110% of preset voltage. Meaning also the voltage drop between the first and last pulse at the maximal number of pulses should be in this range. The pulse duration deviation from the preset value should not be higher than 8%. The proposed tolerances must be fulfilled in case of maximal settings, which are: maximal pulse amplitude, maximal pulse duration, the maximal number of pulses, and minimal pulse repletion rate when the pulse is applied on a typical load for an application that it is made for.

4 Discussion

Electroporation-based therapies have a huge potential for implementation into clinical practice in socioeconomically disadvantaged populations. Electrochemotherapy is easy, quick to perform, and inexpensive. Only suitable room for patient preparation and treatment, an electroporator with suitable electrodes, a physician in charge, and a nurse (and an assistant trained in handling the electroporator) are required for treatment of cutaneous tumors and skin metastases. Because the treatment is safe, with mainly no side effects, a patient can wait for a while in the hospital for the observation, but do not need to spend a night. The treatment can be performed on any part of the body, on different tumor types with the same electroporation device, only appropriate electrodes have to be selected [88]. The SOP [20] should always be followed. Calcium electroporation is even cheaper because calcium is commercially available and regularly used in most hospitals. It can administer by other medical professionals (not only oncologists); it is nonmutagenic and has long durability. Additionally, calcium has an excellent safety profile, both for use in patients and for staff, and would not need administration by staff accredited to administer chemotherapy [27]. However, calcium can only be used for tumors localized in the skin and has to be administered locally.

The main advantages of IRE are apoptotic cell death, the sharp boundary between the treated and untreated areas, and the overall time for the procedure is extremely short in comparison to benchmark ablation treatments. The pulse application lasts only a few minutes; actual time can be calculated from the number of delivered pulses and average heart rate [89,90]. IRE also enables the ablation of tumors in areas previously contraindicated for thermal ablation [90].

Gene therapy by electroporation is a highly efficient method, with delivery efficiency better than many non-viral vectors [91]. It

substantially increases DNA delivery and DNA vaccine potency. The preclinical development of electroporation is focused on tissues that are easily accessible and the application technique is simple and takes only a few seconds after DNA injection. Electroporation is a useful strategy to improve DNA-based vaccination protocols; it stimulates both the humoral and cellular immune responses and if necessary, they can contain several antigen epitopes [49]. Additionally, DNA is more heat stable, easier to produce and store than current vaccines [63]. The transport from the manufacturing to injection into the patient is easier and therefore cheaper, meaning it is perfect for warm climates like the African continent. They do not display an environmental hazard since there is no risk of replication and only contain the antigen encoded [63].

Because different applications require different electric pulse characteristics, and because biological loads vary considerably, specific electroporators are designed for each application. However, it is possible to develop a device that could be used for all presented medical applications. But, the price of such a device would rise, the certification process would be more challenging, and the size and weight would increase in comparison to a single application device. A battery-powered electroporator would enable DNA vaccination even in areas without electricity. Currently, the price of electroporation is relatively high, but custom low budget devices can be developed. Even cheap single-use electrodes, with mass production or multiple-use electrodes with a defined sterilization process, would significantly reduce the price of electroporation.

5 Conclusions

Electroporation is a platform technology, with high potential, it can reduce cost and facilitate treatment procedures. According to the ongoing studies the electroporation-based therapies are safe, with little or no side effects [7]. The clinical data published on electroporation based applications are quite encouraging; therefore we believe, that in the future, electroporation will be indispensable in cancer treatment, infection disease treatment, intracardiac ablation [92], and vaccination [91]. Further development of standard electroporation treatment protocols, regulation of electroporator development, and user training, however, remain essential for successful incorporation into clinical practice. Electroporation-based therapies have a huge potential for implementation into clinical practice also in socioeconomically disadvantaged populations. Currently, the price of electroporation is relatively high, mainly due to costly electroporators and electrodes, but custom low budget devices can be developed. It is not technically difficult to develop an electroporator, more challenging is the quality assurance, because biological loads characteristics vary considerably from sample to sample and even more from tissue to tissue. Therefore, measuring of applied electric pulses is crucial for a successful application.

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Nomenclature

ECT = electrochemotherapy
GET = gene electrotransfer
IRE = Irreversible electroporation
H-FIRE = high frequency-Irreversible electroporation
DNA = deoxyribonucleic acid
FDA = food and drug administration
HV = high voltage
LV = low voltage
WHO = Word Health Organization

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