Towards Standardization of Electroporation Devices and Protocols

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Cell exposure to high-voltage, short-duration electric pulses can lead to temporary formation of hydrophilic pores in the plasma membrane and an increase in the membrane's permeability which consequently increases the transmembrane transport of molecules that are otherwise unable to cross the membrane. This phenomenon, termed membrane electroporation, is currently an applicable technique in different areas such as biomedicine, biotechnology, food technology and environmental applications. Electroporation pulses are generated by pulse power generators known as electroporators and delivered to the cells (in tissue) via electrodes. The objective of this paper is to review and compare characteristics of electroporation applications and equipment described in the literature and/or present on the market. Since there are no specific standards or regulations that specifically refer to the safety of medical devices with intended medical uses for electroporation, we propose guidelines for the design of clinical electroporators and define minimal requirements for their safe and efficient use which can be incorporated within the particular standards in the future. In order to facilitate the comparison of data obtained by different research groups and to enable reproduction of results under the same conditions, we want to stress the necessity of defining the electroporator's output parameters and tolerances of electroporation parameters for electroporation-based therapies.

Electroporation

The Biological Phenomenon

Each biological cell is protected from its surroundings by the plasma membrane which is composed of a two-molecule thick layer of lipids. The plasma membrane would be a mostly impenetrable barrier if there were not various proteins which enable transport of specific molecules across the membrane. However, cell exposure to high-voltage, short-duration electric pulses can lead to temporary formation of hydrophilic pores in the bilayers and increase plasma membrane permeability which consequently causes increase in transmembrane transport of molecules that are otherwise unable to cross the membrane [1]. This phenomenon termed as membrane electroporation/permeabilization can be either reversible, when the cell exposure to electric field is short enough for the cells to fully recover, or irreversible, when their exposure leads to cell death.

Electroporation Applications

Electroporation has become a relevant technique in different areas such as biomedicine, biotechnology, food technology and environmental applications [2]. Reversible electroporation is already a well-established method in medicine, which combined with administration of otherwise low-permeant chemotherapeutic drugs to tumor cells, results in highly efficient local antitumor therapy called electrochemotherapy (ECT). Locally applied short, high-voltage (HV) pulses in the range of few hundreds of volts to few kilovolts increase the permeability of tumor cells membranes to facilitate cellular uptake of chemotherapeutic drugs like bleomycin and cisplatin, thus increasing their cytotoxicity [3]. Electroporation is also used for cell gene transfection, i.e., gene electrotransfer (GET), which is a non-viral gene delivery method that uses reversible electroporation for delivering DNA molecules to cells. Skin and muscle are promising targets for gene delivery, thus GET has been used in many medical applications, including vaccination, wound healing and cancer treatment. On the other hand, the potential of irreversible electroporation in medicine appeared as a nonthermal irreversible electroporation (NTIRE), also termed Pulsed Field Ablation (PFA), which has enabled the ablation of undesirable (malignant or arrhythmogenic) tissue with minimal damage to blood vessels and

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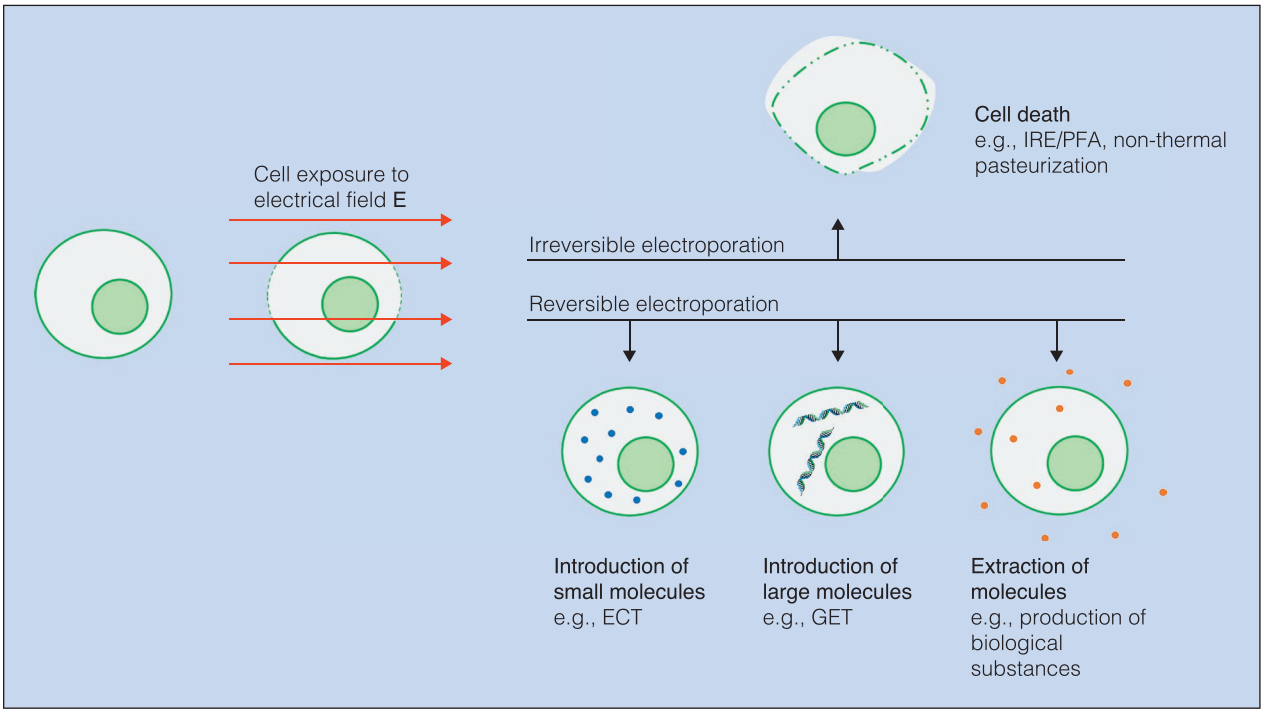

Fig. 1. Various electroporation applications. When exposure of the cell to sufficiently high electric field reaches the cell membrane threshold value, the cell gets permeabilized. Membrane electroporation/permeabilization can be either reversible, when the cell exposure to electric field is short enough for the cells to fully recover; or irreversible, when their exposure leads to cell death. In case of reversible electroporation, during the electroporation process, molecules can be introduced into the cell (electrochemotherapy (ECT), gene electrotransfer (GET)) or molecules can be extracted from the cell.

nerve conduits in the treated area [4]. Furthermore, electroporation emerged in different applications of biotechnology [5], and it is efficiently utilized for heritable genetic modification of microorganisms (electrotransformation), extraction of biomolecules, inactivation of microorganisms, and improving the mass transport in food processes. Some of the applications of electroporation are presented in Fig. 1.

Generating Electrical Pulses

To achieve successful electroporation, cells have to be exposed to sufficiently high electric fields (related also to duration of exposure). Electroporation pulses are electrical pulses, which are generated by pulse power generators known as electroporators and delivered to the cells (in tissue) via electrodes. Electrode geometry and tissue dielectric properties define the electric field intensity. Regardless of the application, electroporators have one common task: electrical pulses generation and delivery.

Electroporation success depends on the parameters of the delivered electrical pulses. The parameters of electrical pulses at the output of commercially available electroporators may vary in shape, voltage/current amplitude, pulse duration, number of pulses in a pulse train, and pulse repetition rate [6]. In electroporation, series of pulses are most commonly used for the treatment. If all pulses in a train/series of pulses are similar, the definition of the characteristics of only a single pulse and its repetition rate is sufficient for defining the treatment. The amplitude of the generated pulses may range from a few tens of volts (e.g., for GET) to a few kilovolts (e.g., for IRE) and even tens of kilovolts for microbial inactivation in liquid food pasteurization, with durations that range from nanoseconds to milliseconds for single pulse or train of pulses with predefined pulse repetition rate. Pulses may be preset for a specific application or precisely defined for a particular patient.

An electroporation pulse generator comprises an HV power supply, a pulse generator, a control unit, a user interface and an output module (Fig. 2). The user interface enables setting pulse parameters as required for a specific application. It is preferable that the device measures the parameters of delivered pulses and generates warnings in case of malfunction and/or incorrect operation.

For generation of electrical pulses, an HV power supply and pulse generator (for pulse shaping) are needed. The output module consists of an output pulse measurement unit and a commutator for switching high voltage pulses to different electrodes (if multiple electrodes are used).

Electroporators for Specific Applications

Electroporators are mainly classified as clinical, industrial or laboratory-based [7]. Electrodes together with the biological sample define the load for the output stage of the electroporator. Thus, classification of the electrodes is according to targeted load, i.e., cells (single-cell chambers, micro-electrodes, macro-electrodes and flow-through chambers) or tissue (plate, parallel needle array, hexagonal needle, finger and adjustable electrodes). Since biological loads as well as the electroporated volume intrinsically differ in different electroporation-based

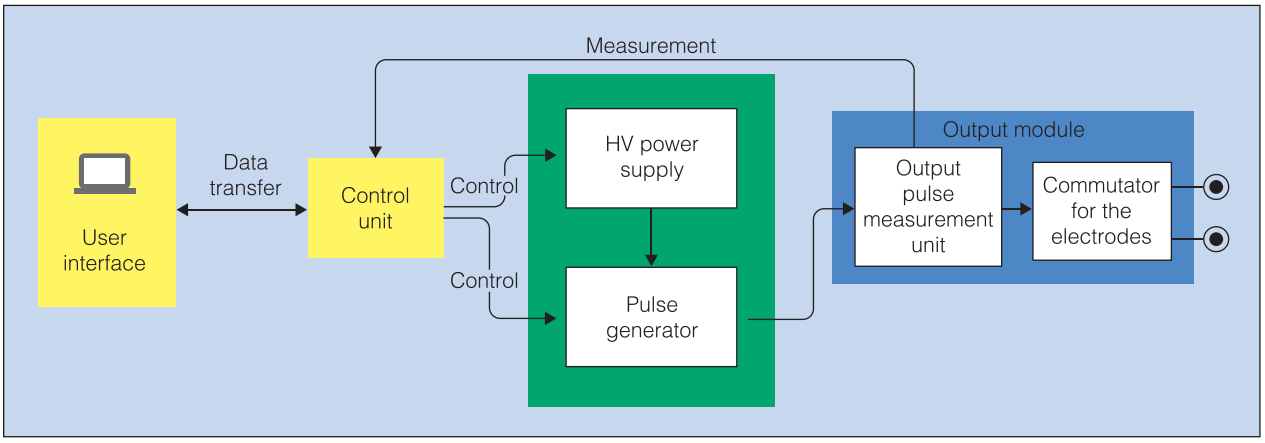

Fig. 2. Block diagram of an electroporator. An electroporator comprises a user interface, to enable setting of the pulse; a control unit; a high-voltage (HV) power supply and pulse generator for generation and forming of electrical pulses; an output module, and in case of multiple electrodes, a commutator for switching the voltage pulses to different electrodes. Measuring of the delivered pulses is preferable and warnings in case of malfunction and/or incorrect operation should be generated.

applications, pulses with specifically predefined parameters have to be used. Therefore, electroporators are often designed and developed for specific applications, where quality and efficiency have to be assured [8].

Clinical Electroporators

Electroporation-based medical/clinical applications such as ECT, GET, IRE and PFA, have so far been focused predominantly on cancer treatments. Electroporators used for medical treatments in clinics, i.e., clinical electroporators, have been developed to implement antitumor therapy using a limited number of predefined settings of electrical pulses in associations with chemotherapeutic drugs (ECT) or foreign substances such as DNA (GET) within protocols based on reversible electroporation or as stand-alone ablation treatment based on irreversible electroporation (IRE). The target tissue (e.g., tumor) should be covered with sufficiently high electric field, which should be above the threshold of reversible or irreversible electroporation 500–600 V/cm local electric field (which often requires pulse amplitudes up to 3000 V and currents up to 50 A), to achieve the desired effect. Therefore, HV pulse generators are needed which should not present potential risk for hazardous event for the patient, operator and nearby devices.

Clinical electroporators are considered to be medical devices, and thus, patient and operator safety must be ensured under both normal and single-fault conditions. Furthermore, they are obligated to meet medical device standards and follow the requirements defined by local medical regulations (e.g., Medical Device Regulation 2017/745 in Europe or Code of Federal Regulations Title 21 in the US) in order to get approval for selling the device on the market, (e.g., certification mark (CE) in Europe or Food and Drug Administration (FDA) approval in the US). In spite of agreements signed between the EU and the US, a clinical electroporator approved for use in Europe cannot be automatically used in the US and vice versa, because each country has different regulatory regimes.

Based on our review, there are only few certified clinical electroporators on the market. The most used clinical electroporators are the Cliniporator and the NanoKnife System. In Europe, the Cliniporator (two different models: Cliniporator EPS02 and Cliniporator VITAE), manufactured by IGEA S.p.A. (Carpi MO, Italy) and used for both ECT and GET, was approved by the Italian notified body and has CE mark. Another certified clinical electroporator in Europe is SENNEX (BionMed Technologies, Germany), which is used only for ECT. Recently, a new CE approved clinical electroporation generator ePORE (Mirai Medical, Galway, Ireland) was developed for simple and reliable delivery of ultra-short electrical pulses up to 250 kHz to enable treatment on an outpatient endoscopy basis. On the other hand, in the US, the NanoKnife System, manufactured by AngioDynamics, Inc. (Queensbury, NY), was approved by the FDA for surgical ablation of soft tissue [9]. Additionally, it is CE marked for Europe and has license approval for Canada for soft tissue ablation. However, it does not have FDA clearance for other treatments and therapies for specific disease or condition.

To help verify the safety of medical devices, electrical safety standards have been established in the US, Europe and other parts of the world. The first medical standard for medical electrical equipment IEC 60601 was published in 1977 by the International Electrotechnical Commission (IEC), which is a family of technical standards whose scope covers the safety, essential performance and electromagnetic compatibility of medical electrical equipment and systems. As of 2011 it consists of a general standard for medical electrical equipment: 60601-1 (EN 60601-1:2006/A1:2013 in EU or IEC 60601-1:2005/A1:2012 in the US), ten collateral standards and about 60 particular standards. Collateral standards (numbered 60601-1-X) define the general requirements for certain aspects of safety and performance, e.g., Electromagnetic Compatibility (IEC 60601-1-2). Particular standards (numbered 60601-2-X) define particular requirements for specific products or specific measurements built into products and add conditions not mentioned in

60601-1 or explain how to simplify implementation of 60601-1 to a particular device type, e.g., cardiac defibrillators (IEC 60601-2-4). In spite of the 60 particular standards, a particular standard for clinical electroporators currently does not exist. Therefore, it will be necessary to define additional rules for manufacturing and safe and efficient use of clinical electroporators as relatively new medical devices in addition to those defined by ISO and EN/IEC standards.

Considering the general standard for medical devices EN/IEC 60601-1, key safety factors that have to be considered in electroporator's design include: voltage and energy limits, adequate insulation, limitation of leakage currents, electromagnetic compatibility requirements as presented in the standard EN/IEC 60601-1-2 and consideration of fault operations while maintaining quality, efficiency and smooth operation of the device. Other standards to be considered for developing clinical electroporators are: ISO 14971 for risk analysis, ISO 13485 for 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 powered clinical electroporator.

Industrial Electroporators

In biotechnology and even more in food processing technology, high-power and high-voltage electroporators are needed due to the requirement to electroporate large volumes of liquid. In food processing, electroporation is more frequently termed as pulsed electric field (PEF) treatment used for food structure modification or liquid food pasteurization. By PEF treatment, permeabilization of biological cells is achieved, mass transfer is improved, and therefore an efficient way for extraction of liquid and valuable substances from cells is enabled. PEF treatment systems are usually composed of a pulse power generator and a treatment chamber.

Depending on the application, a suitable pulse generator has to be chosen with adequate parameters for:

- pulse shape;
- peak voltage, which is highly dependent on the desired application;
- peak current, which is determined by the object and volume being treated;
- geometry of the treatment chamber;
- average power required, depending on the desired processing capacity (kilograms/h or liters/h).

In terms of power requirements, scale-up from several kW for laboratory to more than 100 kW for continuous-flow industrial-level processing was achieved. Therefore, regular average power of contemporary PEF devices ranges between 30 kW and 400 kW [10]. Commercial PEF treatment applications are mostly set up in potato (tuber) industry, fruit juice preservation, and vegetable processing. For juice processing, electroporation treatment systems with continuous flow have already been established with capacity of 8000 liters/h, whereas for potato processing capacity ranges up to 50–80 tons/h.

The use of new processes applied in food industry always requires appropriate process control options and set up of a systematic preventive approach to food safety – Hazard Analysis Critical Control Point (HACCP). HACCP has seven principles that need to be followed, stated in the international standard ISO 22000 FSMS 2011. In the US, the adoption of different technologies in the food processing industry is also subject to the regulation of the FDA, and in the EU it falls under the Regulation EU 2015/2283 for novel foods. Regarding safety of the device, protection against electric shock in case of insulation failure is important when using the device in wet environments. For this reason, wineries for example, are equipped with residual current devices that are responsive to a leakage current of about 30 mA. Furthermore, electromagnetic compatibility according to standards is recommended. Thus, the pulse circuit has to be shielded with metal housing, and mains and leads to the control circuity should be protected against over-voltage [11].

Laboratory Electroporators

For conducting experiments in the laboratory, users can choose between several commercially available laboratory-based electroporators. Choosing the right laboratory electroporator can be crucial for experiments and treatment protocols as some laboratory electroporators have limited range and control over pulse parameters.

An important step to be considered during electroporation is to assure pulse measuring and monitoring because only few electroporators can report and provide accurate measurements. Large variation of load characteristics is another reason to measure. The electrical properties of the sample between the electrodes might affect the current delivered (conductivity versatility). The resistance of the cuvette, for example, can vary depending on the conductivity of the media which can drastically change the required current.

However, in laboratories where experiments are done, oscilloscopes and current probes are often not readily available. Therefore, built-in measurement systems should be provided to be used with laboratory electroporators. The device should be able to perform self-tests to ensure flawless operation and detect single faults. Some use "test" pulses which should be specified and should not affect/change the sample or influence the outcome of the result. Furthermore, the device should be able to interact with the operator to ensure safe and efficient treatment and generation of output pulses, which ensure an effective experiment. The accuracy of measurements should be specified in advance, and measuring and comparison of results during experiments should be reported. Periodic calibrations of the device and equipment need to be made as well as electrode replacement based on predefined intervals. When single-use electrodes or electroporation cuvettes are used, safe disposal after the experiment should be provided due to the chemical reactions that can change the electrical properties of the electrodes in the next experiment. Recently, nanosecond electroporators were introduced and are now being used in laboratory setups. Here, measurement protocols and delivery of the pulses are more challenging. Special attention and more advanced measurement setups are required, as

those electroporators mainly do not have built-in measurement systems.

All of these problems and requirements could be solved with implementation of a specific standard for electroporators based on the application. As laboratory electroporators are not considered to be medical devices, medical device standards do not apply, and therefore, the only solution is to gather all previously mentioned requirements into one standard and agreeing on requirements that will be followed by all manufacturers to enable comparability and reproducibility of research and laboratory results. Additionally, electroporation cuvettes and electrodes should be standardized with defined tolerances and materials used.

Recommendations for Further Research and Standardization

Nowadays, the electroporation industry is growing even faster than before. Taking into consideration the new technologies, treatment protocols, increased research intensity and knowledge, we already have well-established protocols, equipment and promising treatments.

For achieving successful and efficient electroporation, it is necessary to have well defined output pulses and measure them in each treatment or experiment to make sure that the pulses are delivered as requested. Thus, suitable reproduction and comparison of results can be made if necessary. When delivering electrical pulses for electroporation, regardless of the application, it is very important to provide complete reports to enable comparability and reproducibility of the results [4], [12]–[14].

A description of pulses and how the electrical pulses were measured is necessary. The researchers must provide all specifications of the measuring equipment, identify the point of measurement and state if the pulses were delivered and measured in each experiment. Additionally, parameters and complete time-domain waveforms of the pulses should be provided with an appropriate description of the electroporator and electrodes used. For commercial equipment, the name of the company and model should be specified. If the pulse generator is a laboratory prototype or specially manufactured unit, suitable description of the components, electrical configuration, measurement and data acquisition systems should be provided. Lastly, the electric field induced by the delivered pulses inside the biological load should be calculated and/or all data describing the electric field should be listed i.e., electrode shape and their position with respect to the treated sample/tissue.

Currently, we can say that we have a developing market for clinical electroporators and new electroporators designed for specific applications are coming up. However, the absence of industry, laboratory and medical specific standards may eventually become an obstacle for further development of approved electroporation devices and associated equipment. Papers calling for standardization for other applications of electroporation have already been published (e.g., for standardization of IRE techniques and protocols), in which authors propose a set of technical recommendations for the use of IRE for treatment of locally advanced pancreatic cancer [15]. Having a specific standard for each application will simplify the harmonization of all commercial, certified electroporators and improve the safety, quality and efficiency of these devices. Current problems like voltage drop during pulse delivery, unknown pulse parameters, insufficient electrical field, and non-comprehensive reports can be solved by stating limits and recommendations for voltage/current, energy, load, electrodes used, insulation and design.

The standard should define:

- maximum tolerances of generated pulses compared to expected values by considering the plasma membrane permeabilization of the load and technical limitations of electroporation device development;
- how to provide technical specifications of the device, together with conditions under which they are achieved, e.g., to define maximum amplitude of the pulses together with the pulse duration range and load resistance at which it can be achieved;
- pre-pulses (amplitude, pulse duration and exact timing regarding the preset sequence), if used;
- how to implement safety features like galvanic isolation, current, energy and voltage limitations, warnings if the pulse delivery was stopped or limited, or if any other unforeseeable event or malfunction has occurred;
- by which load the electroporators should be tested to ensure effectiveness at specific applications, or to ensure predictable operation or operation within tolerances (this can be quite challenging in case of delivery of nanosecond pulses as pulse reflections can occur due to the dynamics and variability of the biological load, i.e., conductivity increase due to electroporation);
- which electroporation cuvettes and electrodes should be used;
- maximum tolerances of the distances between the electrodes.

The standard should also consider procedures for different materials used and recommend a way of defining a treatment volume and an electric field distribution between the electrodes.

Recommendation for Electrochemotherapy Device Standardization

ECT is an established cancer treatment used in clinics [16] for safe and convenient treatment of cutaneous and subcutaneous tumors following standard operating procedure (SOP) [17], [18]. The pulses are delivered to target tissue via electrodes, which are considered to be medical accessories, used only in combination with a particular pulse generator (mostly used is the Cliniporator EPS02). If the electrodes are placed on the patient's skin (e.g., plate or non-penetrate electrodes), they are considered to be non-invasive medical accessories, used to treat cutaneous tissues. In cases when they are intended to be placed inside the patient's body (e.g., needle electrodes),

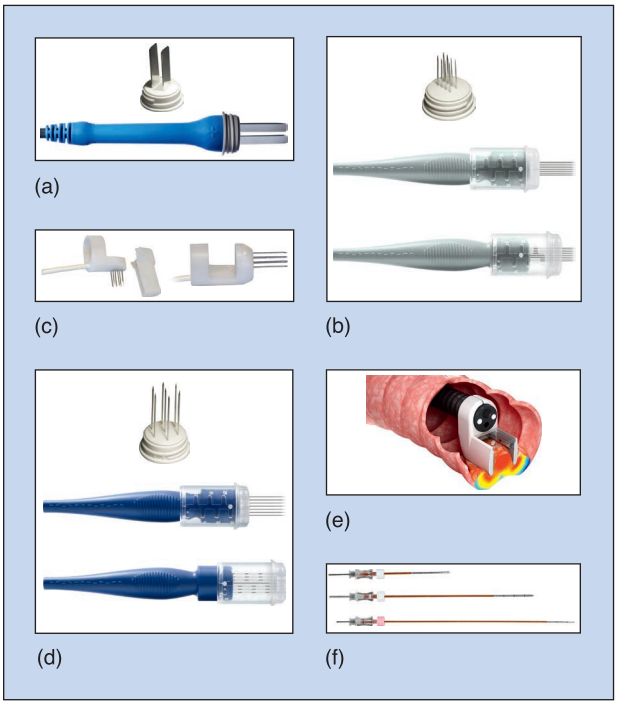

Fig. 3. Different types of electrodes (by IGEA S.p.A.). (a) Plate electrodes. (b) Linear (parallel needle) array electrodes (first top image), adjustable linear needle electrodes with needle-length adjustment with 5 mm increment (bottom two images). (c) Finger electrodes with orthogonal linear needles (left) and longitudinal linear needles (right). (d) Hexagonal needle electrodes (first top image), adjustable hexagonal configuration needle electrodes with needle-length adjustment in 5 mm increments (bottom two images). (e) Endoscopic electrode EndoVE (Endoscopic Vacuum Electrode) which is mounted at the head of an endoscope and utilizes a vacuum source to drag the tissue alongside with the electrode. (f) Individual (long) needle electrodes for variable electrode-geometry (from 2 to 6 electrodes with 16–30 cm long needle and active tip of 3 or 4 cm).

they are considered to be invasive and are used to treat deeper tissues.

The updated SOP [18] defines five types of electrodes (made of stainless-steel) that are commercially available (IGEA S.p.A, Carpi MO, Italy) and can be used together with the Cliniporator depending on the treated area:

Plate electrodes: with 8 mm gap in-between, used for superficial skin lesions (Fig. 3a).

Linear array electrodes: (parallel needle array) that have 2 arrays of 4 needles (with needle length of 10-, 20- or 30 mm), separated by 4 mm distance, used for smaller tumors (recommended to be used for tumors in the facial region) with local anesthesia (Fig. 3b).

Hexagonal needle electrodes: with needle length of 10-, 20- or 30 mm, used for treatment of larger areas, e.g., cutaneous metastases (Fig. 3d).

Finger electrodes: (longitudinal or orthogonal) with needle length of 5- or 10 mm, used for treatment of mucosal tumors, e.g., in the oral cavity (Fig. 3c).

Adjustable electrodes: (linear (Fig. 3b) or hexagonal (Fig. 3d)) allow adjustments in needle length (from 5 mm to 40 mm with 5 mm increments) for better support in treatments of tumors with heterogeneous size.

In addition, the endoscopic electroporation system EndoVe (Mirai Medical, Galway, Ireland) was developed to be used with the ePORE electroporation generator, which is also suitable for the Cliniporator (Fig. 3e). Furthermore, long freely-placeable needle electrodes (Fig. 3f) were introduced, and new minimally invasive laparoscopic expandable needle electrodes are being developed by IGEA S.p.A [3], [16].

Pulse parameters are defined in the SOP as a result of numerous previously conducted studies. For each pulse delivering, 8 square-wave pulses of 100 µs with pulse amplitude of about 1000 V (1000 V up to 1300 V) across an 8 mm distance between plate electrodes should be delivered at repetition rate of either 1 Hz or 5 kHz. ECT with pulse repetition rate of 5 kHz is mandatory for hexagonal needle electrodes because the treatment (delivering 8x12 = 96 pulses) with 1 Hz repetition rate would extend over a prohibitively long time and high-frequency (5 kHz) pulses reduce the number of contractions. Nevertheless, several applications may be needed to cover the whole tumor volume in a single session.

All electrodes which are commercially available and meant to be used with the Cliniporator are for single use for a particular patient and only for a single session (for one nodule or several similar nodules in the same patient). In a case of more nodules of different sizes, more than one electrode type may be needed for a particular patient in the same session.

The galvanic isolation of the output is preferably implemented in the power supply and not in the output module to have accurate measurement of the output signal. Output current and voltage are measured at the output of the pulse generator to implement current, energy and voltage limitations. The SOP should define the maximum expected current or minimal expected resistance of the load. The maximum current of the device should be 10% or 20% higher than the maximum expected current, which is 20 A for the Cliniporator EPS02. SOP defines the maximum treatment voltage as 1300 V/cm voltage-to-distance ratio times 8 mm which is equal to 1040 V. The maximum voltage is defined by the SOP and tolerances and is 1000 V for the Cliniporator EPS02. The maximum energy should be equal to the maximum treatment time, times maximum current, times maximum voltage.

Considering the SOP, for square wave pulses (described by the amplitude and the pulse duration t_{FWHM}, where FWHM is Full Width at Half Maximum, we propose the following tolerances:

- the pulse amplitude between 15% and 85% of FWHM should not rise over or fall below 110% or 90% of SOP amplitude (Fig. 4a);
- the FWHM should not be longer or shorter than ±8% of SOP FWHM;
- delivered number of pulses should be exactly the same as in the SOP and variations of this parameter are not allowed;

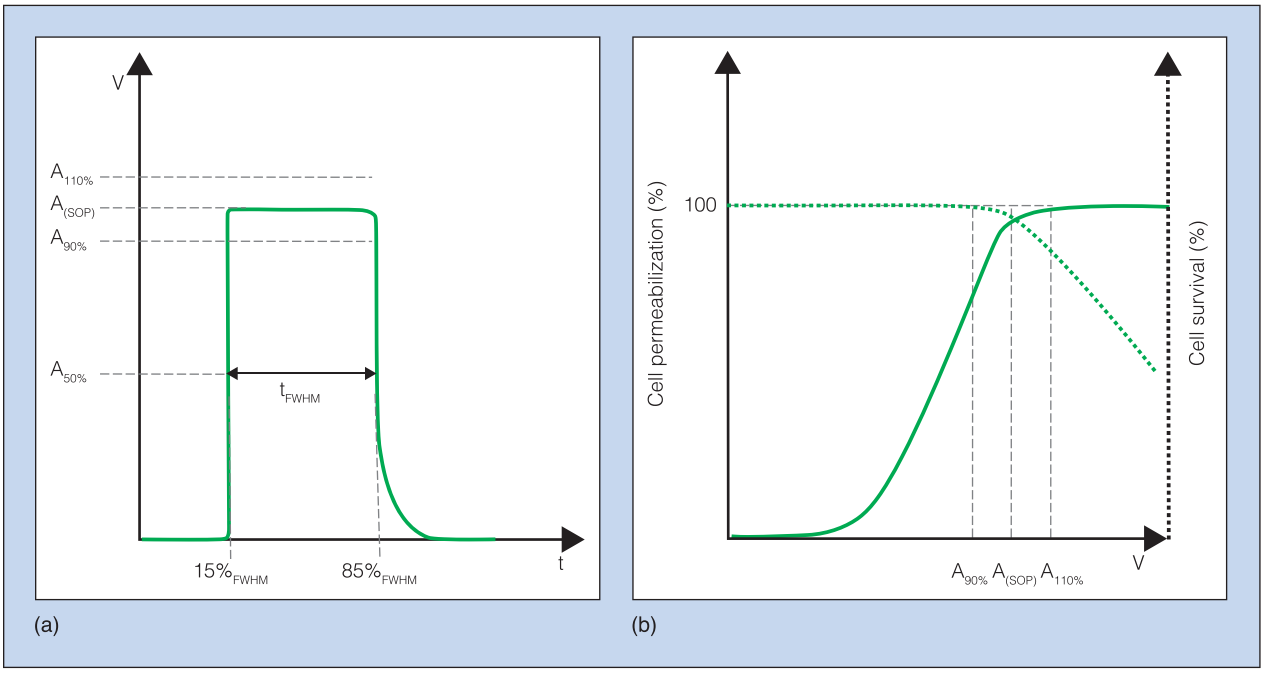

Fig. 4. a) Electroporation square wave pulse: pulse parameters and tolerances. b) Permeabilization curve (solid line) and cell survival (dashed line) with defined tolerances [19]. For amplitudes between 90% and 110% of the defined SOP amplitude will still be possible to achieve efficient treatment. With amplitudes values higher than 110%, cell survival will be increasingly lowered and can lead to IRE while with amplitudes values lower than 90%, the cell permeabilization will decrease and can lead to inefficient tumor treatment.

- pulse repetition rate may deviate from SOP pulse repetition rate (for both options) for maximum ±5%.

For successful ECT it is important to keep within these tolerances, as we calculated them to define the maximum deviations where it is still possible to achieve the desired effect. For example, higher (more than 110% of the amplitude) or lower (less than 90%) values of the SOP amplitude can lead to IRE or insufficient electric field for ECT, respectively. Based on the permeabilization curves (Fig. 2 from reference [19]), even for pulses with the lowest or highest defined tolerances (for pulse amplitude and pulse durations), it will still be possible to stay on the part of the permeabilization curve where the treatment will be efficient (Fig. 4b).

ECT devices should work within the tolerances on zero load and on electronic emulator of ECT load. Operation of the limitation should be tested by the device on power up and conformity by using electronic emulator of biological load.

All electrodes should be manufactured utilizing a biocompatible material, usually stainless-steel. However, materials tested by the requirements stated in the ISO 10993 series of standards for biological evaluation of medical devices that come into direct or indirect contact with biological tissues (parts -1, -5 and -10 are the most important) may be allowed.

Following the SOP, all electrodes need to be for single use. We propose the option of using multiple-use electrodes. In this case, clear instructions for electrode cleaning and maintenance after every treatment should be provided. Moreover, multiple-use electrode replacement on predefined intervals should be stated and provided in the instructions for use.

The allowed tolerances for the diameters of the electrodes, the distances between the electrodes and the length of the needles should also be defined.

Conclusions

With implementation of a specific standard for particular applications, electroporation devices will be safer, treatments more efficient and results more reproducible, which will allow faster and more straight-forward progress of electroporation as well as treatments and therapies based on electroporation. By implementing a particular medical standard for electrochemotherapy electroporation devices, the standard operating procedure will be improved which will result in better and more effective cancer treatment.

References

[1] T. Kotnik, L. Rems, M. Tarek, and D. Miklavčič, "Membrane electroporation and electropermeabilization: mechanisms and models," *Annu. Rev. Biophys.*, vol. 48, no. 1, pp. 63-91, May 2019.

[2] D. Miklavčič, Ed., *Handbook of Electroporation*. New York, NY, USA: Springer International Publishing, 2017.

[3] D. Miklavčič, B. Mali, B. Kos, R. Heller, and G. Serša, "Electrochemotherapy: from the drawing board into medical practice," *BioMed. Eng. OnLine*, vol. 13, no. 1, p. 29, 2014.

[4] A. Sugrue *et al.*, "Irreversible electroporation for catheter-based cardiac ablation: a systematic review of the preclinical experience," *J. Interventional Cardiac Electrophysiology*, Jul. 2019.

[5] T. Kotnik, W. Frey, M. Sack, S. Haberl Meglič, M. Peterka, and D. Miklavčič, "Electroporation-based applications in biotechnology," *Trends in Biotechnology*, vol. 33, no. 8, pp. 480-488, Aug. 2015.

[6] M. Rebersek and D. Miklavčič, "Concepts of Electroporation Pulse Generation and Overview of Electric Pulse Generators for Cell and Tissue Electroporation," pp. 323-339 in *Advanced Electroporation Techniques in Biology and Medicine*, A.G. Pakhomov, D. Miklavcic, and M. S. Markov, Eds. Boca Raton, FL, USA: CRC, 2010.

[7] M. Rebersek, D. Miklavčič, C. Bertacchini, and M. Sack, "Cell membrane electroporation-Part 3: the equipment," *IEEE Electr. Insul. Mag.*, vol. 30, no. 3, pp. 8-18, May 2014.

[8] E. Pirc, M. Reberšek, and D. Miklavčič, "Dosimetry in Electroporation-Based Technologies and Treatments," pp. 233-268 in *Dosimetry in Bioelectromagnetics*, 1st ed., M. Markov, Ed. Boca Raton, FL, USA: CRC Press, 2017.

[9] C. Bertacchini, P. M. Margotti, E. Bergamini, A. Lodi, M. Ronchetti, and R. Cadossi, "Design of an irreversible electroporation system for clinical use," *Technol. Cancer Res. Treamentt*, vol. 6, no. 4, pp. 313-320, Aug. 2007.

[10] S. Toepfl, "Pulsed electric field food processing –industrial equipment design and commercial applications," *Stewart Postharvest Review*, vol. 8, no. 2, pp. 1-7, 2012.

[11] M. Sack *et al.*, "Research on industrial-scale electroporation devices fostering the extraction of substances from biological tissue," *Food Eng. Rev.*, vol. 2, no. 2, pp. 147-156, Jun. 2010.

[12] L. G. Campana *et al*., "Recommendations for improving the quality of reporting clinical electrochemotherapy studies based on qualitative systematic review," *Radiology and Oncology*, vol. 50, no. 1, pp. 1-13, Jan. 2016.

[13] J. Raso *et al*., "Recommendations guidelines on the key information to be reported in studies of application of PEF technology in food and biotechnological processes," *Innovative Food Science & Emerging Technologies*, vol. 37, pp. 312-321, Oct. 2016.

[14] M. Cemazar, G. Sersa, W. Frey, D. Miklavčič, and J. Teissié, "Recommendations and requirements for reporting on applications of electric pulse delivery for electroporation of biological samples," *Bioelectrochemistry*, vol. 122, pp. 69-76, Aug. 2018.

[15] R. C. G. Martin *et al*., "Irreversible electroporation in locally advanced pancreatic cancer: a call for standardization of energy delivery: IRE technique in pancreatic cancer," *J. Surg. Oncol.*, vol. 114, no. 7, pp. 865-871, Dec. 2016.

[16] L. G. Campana *et al*., "Electrochemotherapy–emerging applications technical advances, new indications, combined approaches, and multi-institutional collaboration," *European J. Surgical Oncology*, vol. 45, no. 2, pp. 92-102, Feb. 2019.

[17] L. M. Mir *et al*., "Standard operating procedures of the electrochemotherapy: Instructions for the use of bleomycin or cisplatin administered either systemically or locally and electric pulses delivered by the CliniporatorTM by means of invasive or non-invasive electrodes," *European J. Cancer Supplements*, vol. 4, no. 11, pp. 14-25, Nov. 2006.

[18] J. Gehl *et al*., "Updated standard operating procedures for electrochemotherapy of cutaneous tumours and skin metastases," *Acta Oncologica*, vol. 57, no. 7, pp. 874-882, Jul. 2018.

[19] M. Puc, T. Kotnik, L. M. Mir, and D. Miklavčič, "Quantitative model of small molecules uptake after in vitro cell electropermeabilization," *Bioelectrochemistry*, vol. 60, no. 1-2, pp. 1-10, Aug. 2003.

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