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Recommendations and requirements for reporting on applications of electric pulse delivery for electroporation of biological samples

Cemazar M.[a,b], Sersa G.[a], Frey W.[c], Miklavcic D.[d], Teissié J.[e,*]

[a] Department of Experimental Oncology, Institute of Oncology, Ljubljana, Zaloska 2, 1000 Ljubljana, Slovenia
[b] Faculty of Health Sciences, University of Primorska, Polje, 42, 6310 Izola, Slovenia
[c] Karlsruhe Institute of Technology (KIT), Institute for Pulsed Power and Microwave Technology (IHM), 76344 Eggenstein-Leopoldshafen, Germany
[d] University of Ljubljana, Faculty of Electrical Engineering, Trzaska 25, 1000 Ljubljana, Slovenia
[e] Institut de Pharmacologie et Biologie Structurale, IPBS, Université de Toulouse, CNRS, UPS, Toulouse, France

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ABSTRACT

Electric field-induced membrane changes are an important approach in the life sciences. However, the developments in knowledge and translational applications face problems of reproducibility. Indeed, a quick survey of the literature reveals a lack of transparent and comprehensive reporting of essential technical information in many papers. Too many of the published scientific papers do not contain sufficient information for proper assessment of the presented results. The general rule/guidance in reporting experimental data should require details on exposure conditions such that other researchers are able to evaluate, judge and reproduce the experiments and data obtained. To enhance dissemination of information and reproducibility of protocols, it is important to agree upon nomenclature and reach a consensus on documentation of experimental methods and procedures. This paper offers recommendations and requirements for reporting on applications of electric pulse delivery for electroporation of biological samples in life science.

1. Introduction

This manuscript outlines a proposal for defined nomenclature and guidance for reporting of materials and methods related to the use of electroporation of biological samples in the life sciences, for both in vitro and in vivo applications. This paper is presented in support of the Electroporation-Based Technologies and Treatments courses (EBTT) (http://2017.ebtt.org/) that supply participants with sufficient theoretical background and practical knowledge for effective use of electroporation in their working environments. This work is intended as a complete set of advice and suggestions on the information that should be included in scientific papers to fully describe the results.

As a general rule/guidance in reporting, experimental data should contain details on exposure conditions such that that other researchers are able to evaluate, judge and reproduce the experiments and data obtained. This type of reporting is necessary for future systematic reviews and/or meta-analyses, which are studies that systematically assess previous research and derive conclusions that cannot be extracted from single studies [1–4]. The outcomes of meta-analyses can lead to further advances in the field of electroporation-based technologies [2,5]. Offering adequate description of materials and methods used in the study presents an apparent contradiction with the trend towards papers that are more concise and shorter in length, but many journals now offer publication of additional (Supplemental) material online. We should all embrace these options.

Such a conclusion on the need for recommendation and guidance on reporting materials and methods is shared by many practitioners [6–8]. Common agreement exists that recommendations are needed to improve the consistency and quality of reporting in life science articles [9–11]. The Global Biological Standards Institute (GBSI) presented a report making a case for biological standards in the life sciences. In interviews in the life science community, working with irreproducible data and/or results was emphasized as a serious issue. The conclusion of the GBSI was that "there is a need for more well-defined and consistently used standards, both material (reference reagents and chemicals) and written (optimal practices and methodologies). This need is urgent because life science research is increasing in its complexity. Due to economic pressure, conclusions must be rapidly available for translational opportunities, mostly for medical applications. To facilitate interpretation and improve the reliability of published results in life and health sciences, we need to report key protocol details more systematically, examine the statistics more closely and offer additional ways for authors to be transparent about these matters. If researchers detailed their exploratory studies more accurately, late-stage trials would be better planned and executed" [12].

The current recommendation guidelines target all electroporation-based applications of delivery of electric pulses to cells and tissues (in which most work is performed with batch processes and electrodes are held at a fixed position during pulse delivery). This scope includes

* Corresponding author.
E-mail address: justin.teissie@ipbs.fr (J. Teissié).

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biomedical pre-clinical applications of electroporation. This manuscript is also a result of the discussions within the COST TD 1104 action "European Network for Development of Electroporation Based Technologies and Treatments", starting with comments from the steering committee in Salerno in 2012, where members noticed that in many presentations, participants did not supply sufficient information for other researchers to properly assess the results. A workshop was subsequently organized in Copenhagen (2014) to discuss issues related to terminology and reproducibility issues (http://www.electroporation.net/Events/COST-TD1104-Management-Committee-Meeting-and-WG-Meeting-Symposium-March-27-28th). Presenters and discussants identified major problems related to reporting of electric pulse delivery for electroporation of biological samples. Finally, during the 1st World Congress on Electroporation held in Portoroz, Slovenia during September 6–10, 2015 the decision was made to prepare recommendation papers specific to electrochemotherapy [13], food processing [14], and life sciences. Selected critical issues were raised in discussions during the COST TD 1104 action [15] that should be further highlighted

a- Emphasis on good practice in experimental design
b- Modeling based on data obtained on selected reference cells and tissues
c- Unification of terminology or a need to supply clear-cut descriptions of the wording.

In this paper, the above principles for reporting are presented using examples and critical details discussed in several Appendixes. We have conceptualized this recommendation paper to make it as short and comprehensive as possible. Therefore, more extensive descriptions are added in an appendix. Furthermore, a one-page summary has been prepared and is included at the end of this paper. This summary can be used as a checklist for authors when writing manuscripts. This work is in line with CONSORT (Consolidated Standards of Reporting Trials) that offers "a standard way for authors to describe how trials are designed, analyzed and interpreted" [12].

2. Terminology

The definition of terms and their explanations are often missing, and therefore, persistent confusion and misuse of terminology is rife in the literature. The consequence is poor reproducibility of results, which is a critical problem for the description of the biological effects of pulsed electric fields, a scientific discipline that is both multidisciplinary and interdisciplinary. Different terminologies are found in physical chemistry and in life science that allow confusion in the reports.

Pulsed electric field treatment (PEF treatment, electropulsation, electroporation) is the process of exposing cells in suspension or tissue to electric pulses. Electric field exposure can be applied via direct, capacitive or inductive coupling [16–18]. This paper focuses predominantly on research that applies direct coupling (direct conductive contact of the electrodes with the sample). With respect to cell suspensions and plant tissue in suspension, this process can be applied either in batch or in continuous mode [19–21]. The major consequence of PEF treatment is permeabilization of the plasma membrane (enhanced transmembrane transport). One underlying hypothesis of the basic effects of membrane permeabilization is formation of defects (known as "pores", hence electroporation) [22]. An accurate definition is required because increasingly more new expressions are used (EP (electropulsation, electropermeabilization, electroporation), ECT (electrochemotherapy), PEF (pulsed electric field), electrogene therapy, GET (gene electrotransfer), electroextraction, electrofusion, electrochemoembolization). As an example, we use electrochemotherapy because this term is already widespread. As an analogy, one could use chemotherapy locally potentiated by electric pulses. We recommend using gene electrotransfer (GET) rather than electrogene transfer/therapy (EGT), although the latter is currently more frequently used.

Increased membrane permeability should refer to a given X molecule, which may be small or large. In most experiments, it is the transport of the given X molecule that is assayed, which is different from increased membrane conductivity, i.e., increased current and increased suspension conductivity due to increases in ionic leakage and heating [23,24], but it remains an associated phenomenon, where transport of X is the key parameter in the assay [22,25].

3. Physical parameters

It is important to standardize the reporting information related to what is currently referred to as a PEF session, such as a train of pulses or pulsing frequency vs. pulse repetition frequency. When these terms are used, it is unclear as to the intended meaning.

Certain PEF parameters are under direct control of the settings of the electric pulse generator and the definition of the applicators (electrodes). A key definition is that one should report the voltage actually applied between/delivered to the electrodes. The electric field is a more complex parameter that depends on the geometry of the experimental system and on the heterogeneity of the sample (tissue or cell density) in terms of conductivity and permittivity [26–29]. Furthermore, whether single or multiple pulses were delivered should be clearly reported. Electropermeabilization is a dynamic process in which local time-dependent changes in the tissue conductivity occur [30]. This process results in a redistribution of the field during the pulse application [31,32].

3.1. Electric pulse generators

The technology that supports pulse generators is complex [33,34]. The type of generator should be described with the specification of whether it is a commercial model or an in-house/built set-up. Because few pulse generators report/offer reliable and accurate measurement of U (voltage) and I (current) [35], monitoring of the pulses with digitized recording (and display of the graph) is an important step in ensuring that pulse delivery was obtained as requested from the settings of the generator. The electrical properties of the sample between the electrodes might affect the current delivered (conductivity change). The electric charge stored in the generator might fall short of that needed and affects the profile of the voltage (in the case of long pulses, i.e., tens of ms, high frequency). It is recommended that treatment parameters and experimental profiles, e.g., voltage and current waveforms, are stored for later re-evaluation. A precise description of this step is required [36]. Authors should report how the voltage (and current) was measured, i.e., where and with which instrument(s), and a schematic drawing of the measurement circuit/setup is helpful.

3.2. Electrodes

Because the electric field at a point E(x, y, z) is equal to the negative gradient of the electric potential, it is strongly dependent on the geometry of the electrodes to which the voltage is applied [37]. Thus, the geometry of electrodes and the sample/tissue treated should be described. The design of the electrodes (cuvette, plate, needles, wires, etc.), including the composition (material) of the electrodes, should be stated because electrochemical reactions occur during application of the electric pulses that could affect the sample and consequently the results [38,39].

However, if the equipment is a manufactured product, the authors should list the reference if the requested info is provided freely on the web. However, the details on the placement and penetration of needle electrodes should also be supplied to enable reproduction of the experiment (Appendix 1) and/or to evaluate the electric field distribution via numerical modeling [40] for comparison with other studies.

For use of arrays (using hexagonal electrodes or other types of multi-array electrodes), a complete description of the complex geometry should be included together with the sequence of pulses delivered

[41] because it was stipulated that the sequence of pulses might have a significant influence on the outcome of treatment [42–44]. This point is highly important in the case of non-penetrating electrodes or in applications in which the limited depth of penetration to the skin presents a major advantage by focusing on the effects on the epidermis [45–47].

3.3. Pulse duration

A clear definition of the temporal pulse parameters is required, such as the duration of square wave pulses or the decay time constant for exponentially decaying pulses generated by capacitor discharge systems [17].

The decay time constant characterizes the time elapsed until the pulse voltage value decays to 1/e = 0.3678 of the pulse maximum (Annex 2). Determination of the decay time constant is only applicable for resistive and capacitive discharge circuits. For bell-shaped voltage waveforms (caused in most instances by a non-negligible influence of the inductance of a discharge circuit), the pulse should be specified by stating either the amplitude, rise-time and fall time of the pulse or the half width and pulse amplitude (Appendix 2).

The shape of the pulse should be described because it is known to affect the extent of membrane permeabilization (exponential decay, square wave or pseudo-square wave) [48]. Selected information on the voltage pulse rise-time should be included whenever possible because it controls the charging time of the induced transmembrane voltage (Appendix 2, Appendix 3). Information on the pulse polarities in a train should be supplied because they are known to control the biological response [49].

Furthermore, the rise-time of a measurement system T_{rMsys} is given as Tr of the step response of the system (response to an infinite fast-rising pulse). The bandwidth B and rise-time of the measurement system TrMsys are related via the bandwidth-rise-time analogy: $B\,T_{rMsys} = 0.35$ (Gaussian systems). The real rise-time of a pulse T_{rReal} is the vector subtraction of the rise-time of the measurement system TrMsys from the rise-time $T_{rDisplay}$ determined at the screen of the oscilloscope: $T_{rReal} = Sqrt(T^2_{rDisplay} - T^2_{rMsy}s)$ (Fig. 1).

For experiments with nanosecond pulses, it is recommended to specify the measurement system used and to also list either the bandwidth or rise-time of the system. This information becomes important because pulse duration can be shorter than or of the same order as the membrane charging time constant. The authors should also mention either the measured rise-time (as commonly observed in publications if "rise-time" is mentioned) or the real pulse rise-time according to above considerations [2]. The rise-time of a pulse is most often defined as the time between 10% and 90% of the pulse amplitude [17]. Traces of the voltage (and current) should be presented or given as supplementary material whenever possible because it has been demonstrated that pulse shape might have a significant effect on the observed outcome (e.g., cancelation effect) [49,50].

When a train of pulses is delivered, the number of repetitive pulses and the pulse repetition frequency (or delay between pulses) should be reported [51]. Memory effects by the cell are present as the field effect is applied on a different membrane organization when resealing is not fully completed [52]. This effect is known to play a direct role in the electropermeabilization process and to simultaneously support a trivial (but damaging) role by increasing the Joule heating [53].

Complex trains, i.e., combinations of different pulses, are currently popular for gene electrotransfer in preclinical applications such as in the use of bipolar pulses, which are a combination of high voltage and low voltage pulses [54–57]. In these applications, the sequence should be reported with all delays and other parameters (Fig. 2).

Many studies that use commercial devices do not supply the parameters of pulses due to a lack of data from the manufacturer [35,58]. Instead, the authors list the "program" or "sequence" that they have selected on the device, which should be documented elsewhere and referenced in the manuscript, although this practice should be avoided. Whenever possible, actual physical parameters/pulse parameters and electrodes should be described. If this information is not available, the program and catalog number of the device, electrodes, reagents, buffer and program/sequence used should be listed.

4. Statistical analysis

Raw data or average/mean median as a measure of central tendency should be listed depending on the data distribution (normal or otherwise) as well as a measure of data spread such as standard deviation, percentiles, etc. The number of cells, sample volume, and repetitions of the experiment should also be documented. When normalizing data, i.e., reporting results in %, care should be taken to avoid reporting percent of a percent. It should be absolutely clear as to what reference the results were normalized against (Appendix 4). Information on the software package used in statistical calculations and graphical representation should also be listed.

5. Biochemical and biological parameters

Most journals offer complete instructions for authors, and these should be carefully followed (for example: https://www.elsevier.com/journals/bioelectrochemistry/1567-5394/guide-for-authors).

5.1. Chemicals

Manufacturer references are necessary (purity and origin are decisive parameters in many cases). The sizes of the molecules, the vehicle in which the chemicals are dissolved, the concentrations used, whether the solutions were freshly prepared for each experiment and the incubation times before and after the electroporation procedure should all be stated.

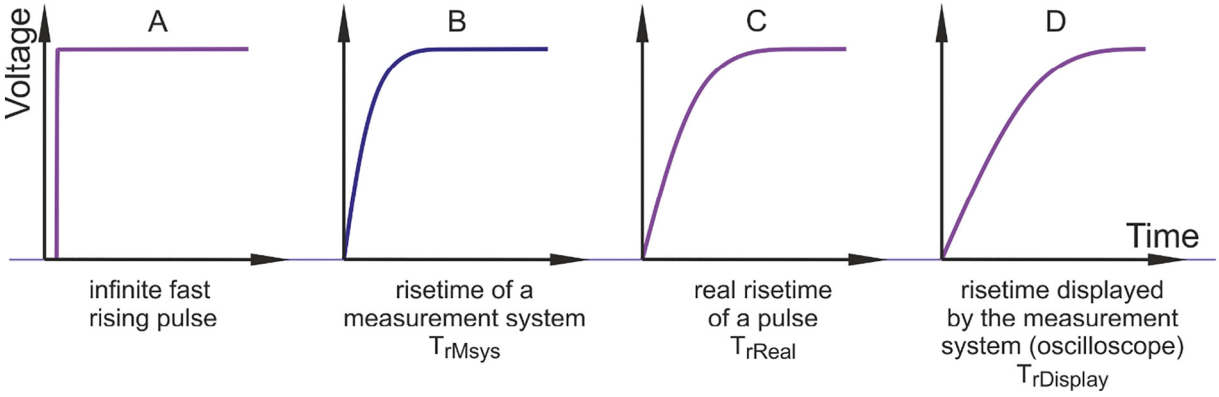

Fig. 1. Influence of the measurement system properties on determination of the pulse rise-time. An infinite fast pulse (A) is displayed by a measurement system of limited bandwidth with a longer rise-time (B). Thus, this measurement system also displays larger values of rise-time (D) when measuring real pulses (C). The rise-time of the measurement system T_{rMsys} (B) is given as T_r of the step response (A). The rise-time $T_{rDisplay}$ observed at the screen of the oscilloscope (D) is the convolution of T_{rMsys} with the real rise-time of a pulse T_{rReal} (C) that is actually delivered on the sample.

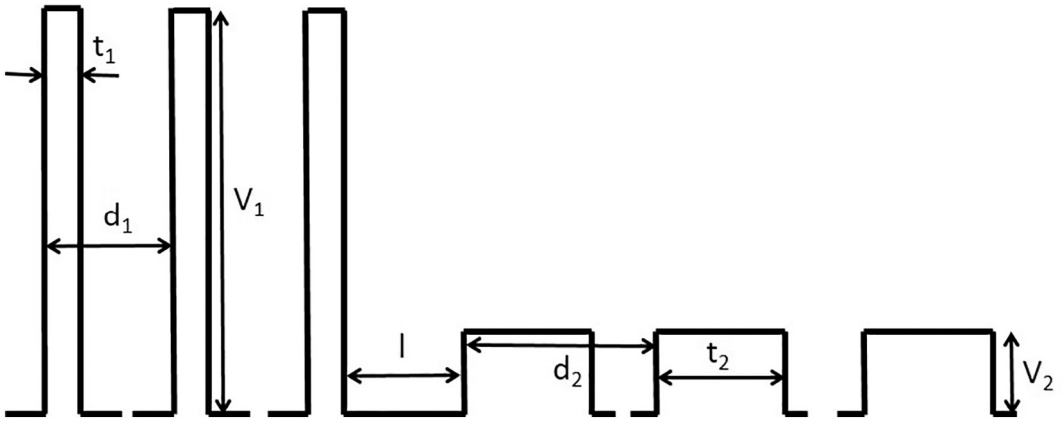

Fig. 2. Example of how to report a complex sequence of pulses. 3 short high voltage pulses are followed by 3 longer low voltage pulses. t_1 and t_2 – pulse duration; d_1 and d_2 – interval (delay) between the pulses; V_1 and V_2 – amplitude of pulses; l - time lag between pulses of different amplitude. The number of every type of pulse should also be stated. As an example of complex pulse sequences, see [59].

In the description of pulsing and post-pulse incubation buffers, in addition to pH, authors should report the osmolarity and conductivity (in S/m), including how and with what (instrument, protocol) these parameters were measured because these factors are known to affect the extent of permeabilization, the response of cells and the contributing effects due to Joule heating. After exposure of cells to electric pulses, post-treatment conditions should also be stated. These conditions include the incubation time, temperature (of the electroporation buffer or medium as well), and addition of fetal bovine serum or other additives (e.g., polymers) that affect cell behavior (viability, survival) as a result of the exposure to pulsed electric fields.

One problem is the use of patented products from companies for which such information is not available, except in the patent protecting the product. In such cases, reference to the patent should be supplied.

5.2. Cells

A complete description of cell systems, as listed below, is required because this information appears to be a decisive parameter in the biophysical response of the cells exposed to electric pulses. The full name of the cells, name of the supplier, catalog number when relevant, confirmation of authentication of the cell line and a statement on mycoplasma testing should also be included. Such reporting on cell lines is increasingly required by most journals in the field of life science. A description of cell cultivation (including the reference of the producer and, if relevant, the size of the plastic dishes), number of passages and whether the cells used in experiments were collected from the exponential growth phase should be presented. Furthermore, preparation of cells for treatment should include the composition of the buffer or medium, the state of the cells (attached vs. suspension), trypsinization or scraping procedure and the cell concentration. Concentration of the cells is highly important because it might affect the sample conductivity during pulse application [23] and the field homogeneity when it is high [60,61]. Additionally, the availability of drug to be introduced into cells can be reduced [29].

5.3. Plasmid DNA (pDNA) and other nucleic acid molecules

When using nucleic acids (including peptide nucleic acid), several parameters should be considered. For plasmids, a reference should be given for the producer if commercially available plasmids are used or to the patent number if the plasmids are proprietary. Otherwise, a map of the plasmid should be supplied stating the size of the plasmid, the promoter used, the therapeutic or reporter gene inserted and the selection marker. The size of the plasmid is particularly important because in the case of smaller plasmids, higher numbers of molecules are present in the suspension if only the concentration (in mg/ml) is stated. Therefore, the molarity of the plasmid should also be included. The preparation procedure should be clearly described, including a statement on endotoxin presence (testing) and verification of the plasmid size and purity. The vehicle in which the plasmid is dissolved should be given. For siRNA, miRNA and other small nucleic acids, including peptide-nucleic acids (PNA), the sequence should be supplied. All information listed for the plasmid DNA, (concentration, molarity, vehicle data) should also be listed [62].

The use of control plasmids and other control nucleic acids is essential. The control plasmid should be devoid of the therapeutic gene and ideally should have the same size as the therapeutic plasmid but with a scrambled sequence. Specifically, nucleic acids represent foreign DNA to cells (in vitro and in vivo) and can cause several different biological cellular responses with respect to its composition (e.g., the presence of bacterial CpG sequences) [63–65]. This information is required to supply a clear view of the possible biological responses.

6. Microscopy

Permeabilization and reporter gene expression procedures are routinely assayed using a fluorescence approach, and fluorescence microscopy reports a cellular description of the results. A precise description of the microscope type (phase contrast, fluorescence, confocal, biphoton) is required together with a precise description of the different elements (references of the objectives, light source, etc.) (Appendix 6). The size and number of pixels of the camera should be given. The conditions of the experiments should also be reported, as the integration time for the camera, continuous or shuttered illumination. When possible, pictures should be displayed using the same imaging protocols to allow direct comparison. The procedure for image analysis and the software used should also be included [66].

7. Animals in electroporation-based biomedical research

When animals are used in experiments [67], Directive 2010/63/EU and/or U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals guidelines should be followed. The experiments should be conducted according to the following 3R principle: replacement, reduction and refinement of the use of animals. The statement on ethical approval of the study, including the approval number, should be supplied.

A description of the animals (species, strain, sex, and age), the supplier, and the animal housing conditions should be included. In addition, if transgenic animals are used, their characteristics should be listed. The number of animals included in each experimental group and the number of repetitions of the experiments should not be left out (http://www.nih.gov/about/reporting-preclinical-research.htm). In addition,

the paper should state whether and how randomization was performed and whether certain measurements or analyses were applied in a blind fashion (e.g., histological analysis or tumor measurements). If possible, animals of both sexes should be used to avoid gender differences in response (Appendix 7).

7.1. Injection of substances and application of electric pulses in animal experiments

As previously described for in vitro experiments, information on substances injected into laboratory animals should follow specific guidelines. The producer of the chemical, drug, plasmid DNA, etc. should be stated. In cases in which the general name of the product is not sufficiently specific (e.g., lipopolysaccharide or LPS), the catalog number should also be supplied. Information on the route of administration and speed of injection is important. Depending on the route of administration, the dose (in mg/kg for intravenous and intraperitoneal injection) or the concentration (in mg/ml for intra-tissue injection, i.e., intramuscular, intratumoral, intradermal, subcutaneous, etc.) should be stated. The injection volume and speed of injection [68], including the size of the needle, should follow the guidelines for the specific route of administration and specific animal species.

When combining the administration of substances with application of electric pulses, the time interval between these two applications should be specified. The term "immediately" should be avoided because it is prone to different interpretations. Note also that delivery of electric pulses affects blood perfusion [69] and can result in different efficacies of injected substances [70].

Associated with the description of the electrodes, it should also be reported whether the electrodes are invasive or noninvasive. Furthermore, if shaving or depilation is required, the method should be described. For tissue electroporation, is also essential that a detailed description of the electrode placement is included. For example, in muscle gene electrotransfer, due to the shape of muscle cells, the position of the electrodes can play a major role in the effectiveness of transfection. When the electric field is oriented perpendicular to muscle cells, the transfection is higher because a larger area of the membrane of muscle cells is exposed to the electric field above threshold for effective transfection [71]. In addition, if conductive gel is used during the treatment to ensure contact between the tissue/skin and electrodes, this item needs to be listed, and details and abundance of use should be described. The producer/manufacturer of the gel and the product number should be reported, including the electrical conductivity of the gel if possible. If the conductivity is not supplied by the manufacturer, the authors should report how the conductivity was measured/determined [72,73].

7.2. Preparation of animals: anesthesia

Another important aspect is the use of anesthesia and analgesia, which can greatly affect the results from measurement of biological effects. The method of anesthesia and analgesia should be selected according to established procedure and should comply with relevant legislation. A considerable amount of relevant literature in this field is available and should be consulted [74–77].

7.3. Reporting results of animal studies

Depending on the type of study, the data pertinent to the aim of the study should be presented. However, certain general data related to the animals should also be reported, such as data on adverse effects. These data should include bodyweight change as a general index of toxicity and whether specific damage to the tissue used in the experiment occurred. Furthermore, the use of non-invasive imaging techniques such as ultrasound imaging, luminescence and fluorescence imaging, CT, MRI, etc. should be implemented whenever possible to comply with the 3Rs (refinement, reduction, replacement) [71,78].

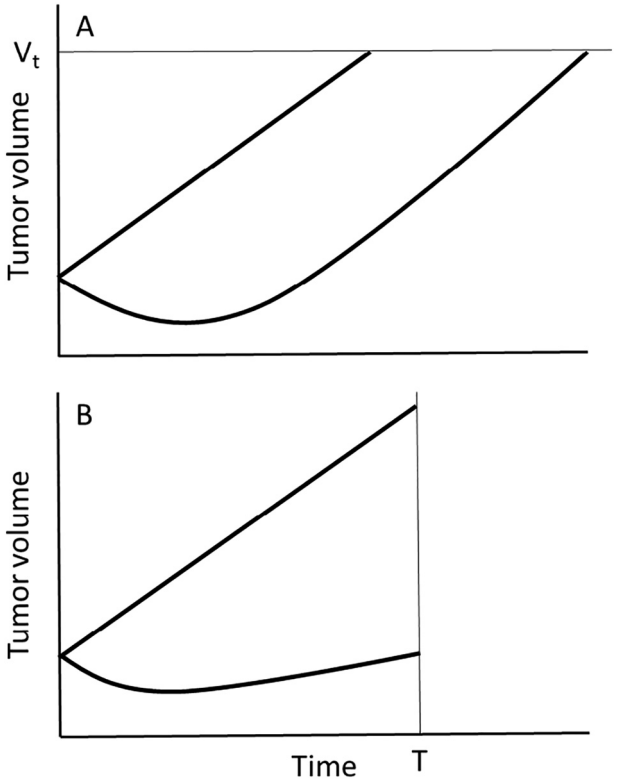

Fig. 3. Schematic representation of growth curves. A growth curves of control and treated tumors. The curves should be drawn to specific volumes (V_t) in all groups, and not only to a specific time point (T) as in B, because the information on possible tumor regrowth in the treated group is therefore missing.

If working with tumor models, the method for measurement and calculation of the tumor volume should be specified. Growth curves, which state the increase in volume or diameter over time, should be followed to the specific target volume V rather than at equal time (Fig. 3A, B). If all animals in the control and treated groups are sacrificed at the same day, then the growth of tumors is followed only to specific time point T. In this case, if the treatment results in regression of the tumor, the data on possible regrowth of the tumors are lost (Fig. 3B).

8. Conclusion

Reporting on applications of electric pulse delivery for electroporation of biological samples in the life sciences requires a description of many factors stemming from the multidisciplinary aspect of electroporation (electropermeabilization). As general guidance in reporting experimental data, a checklist is supplied to aid and guide the authors of research papers in writing and eventually improving the reproducibility of reports.

Conflict of interest

The authors have no relevant financial interest or financial conflict apart from those disclosed.

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CHECKLIST

Recommendations and requirements for reporting electric pulse delivery for electroporation of biological samples

General remarks

The Guidelines for Authors for the journal should always be carefully observed.

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Treatment information:

- Study design, protocol details
- Drug and chemical details (producer, dose, concentration, route of administration)
- Electroporation protocol, time interval between drug and chemical administration and electroporation protocol (number of electric pulses applied)
- Technical details of the electric pulse generator, including type, manufacturer and version of software, if applicable
- Information on the pulsing chamber
- Information on the electrodes (material, size and shape), according to tumor type
- for the nanopulses: report on impedance adaptation and connectors
- Inclusion of a report on electrical parameters (n, T, U, I, f, polarity)*

* Legend: n = number; T = duration of pulses; U = voltage amplitude applied; I = current measured; f = pulse repetition frequency

Culture conditions

- Reference to the cell type and its source.
- □ Initial inoculum
- □ Growth medium composition
- □ Growth temperature
- □ Incubation time
- □ Growth phase (exponential or stationary)

Pulsing buffer

- □ Conductivity and osmolarity of the medium
- □ Temperature

Recovery conditions

- □ Time and storage conditions between treatment and plating
- □ Composition of the recovery medium
- □ Incubation time
- □ Incubation temperature

- Reference to the animals used in experiments, strain, breeder, license
- Type of anesthesia for preclinical studies
- Tumor type, site of implantation, number of tumor cells injected, volume of injection, size of the tumor at the beginning of treatment

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Treatment outcome assessment:

- Methods of response assessment (viability, biochemical responses)
- Type of endpoint for assessment of effectiveness in vivo

.....

Analysis and interpretation of results:

- Summary of trial endpoints
- Interpretation of results
- Future research directions

Appendix A. Annex

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioelechem.2018.03.005.

References

[1] B. Mali, T. Jarm, M. Snoj, G. Serša, D. Miklavčič, Antitumor effectiveness of electrochemo-therapy: a systematic review and meta-analysis, Eur. J. Surg. Oncol. 39 (2013) 4–16.
[2] T. Batista Napotnik, M. Reberšek, P.T. Vernier, B. Mali, D. Miklavčič, Effects of high voltage nanosecond electric pulses on eukaryotic cells (*in vitro*): a systematic review, Bioelectrochemistry 110 (2016) 1–12.
[3] M. Borenstein, L.V. Hedges, J.P.T. Higgins, H.R. Rothstein, Introduction to Meta-Analysis, Wiley, West Sussex, UK, 2009.
[4] J.P.T. Higgins, S. Green (Eds.), Cochrane Handbook for Systematic Reviews of Interventions, 1st EditionWiley-Blackwell, Chichester, England, Hoboken, NJ, 2008.
[5] A.B. Haidich, Meta-analysis in medical research, Hippokratia 14 (Suppl. 1) (2010) 29–37.
[6] N.S. Blow, The key to good science: increased transparency, BioTechniques 61 (2016) 221.
[7] F.S. Collins, L.A. Tabak, NIH plans to enhance reproducibility, Nature 505 (2014) 613.
[8] B. Pulverer, Reproducibility blues, EMBO J. 34 (2015) 2721–2724, https://doi.org/10.15252/embj.
[9] M. Baker, 1,500 scientists lift the lid on reproducibility, Nature 533 (7604) (2016) 452–454, https://doi.org/10.1038/533452a.
[10] N.S. Blow, A simple question of reproducibility, BioTechniques 56 (2014) 8, https://doi.org/10.2144/000114117.
[11] J.C. Lopez, Raising standards, Nat. Med. 19 (2013) 508, https://doi.org/10.1038/nm0513-508.
[12] E. Dolgin, Publication checklist proposed to boost rigor of pilot trials, Nat. Med. 19 (2013) 795–796.
[13] L. Campana, A.J.P. Clover, S. Valpione, P. Quaglino, J. Gehl, C. Kunte, M. Snoj, M. Cemazar, C.R. Rossi, D. Miklavcic, G. Sersa, Recommendations for improving the quality of reporting clinical electrochemotherapy studies based on qualitative systematic review, Radiol. Oncol. 50 (2016) 1–12.
[14] J. Raso, W. Frey, G. Ferrari, G. Pataro, D. Knorr, J. Teissie, D. Miklavcic, Recommendations guidelines of information that should be reported in studies on microbial inactivation and improving mass transfer by pulsed electric fields, Innovative Food Sci. Emerg. Technol. (2016)https://doi.org/10.1016/j.ifset.2016.08.003 (accepted).
[15] D. Miklavčič, Network for development of electroporation-based technologies and treatments, J. Membr. Biol. 245 (2012) 591–598.
[16] R. Korenstein, D. Somjen, H. Fischler, I. Binderman, Capacitative pulsed electric stimulation of bone cells. Induction of cyclic-AMP changes and DNA synthesis, Biochim. Biophys. Acta 803 (4) (1984) 302–307.
[17] M. Reberšek, D. Miklavčič, C. Bertacchini, M. Sack, Cell membrane electroporation – part 3: the equipment, IEEE Electr. Insul. Mag. 30 (3) (2014) 8–18.
[18] V. Novickij, J. Dermol, A. Grainys, M. Kranjc, D. Miklavčič, Membrane permeabilization of mammalian cells using bursts of high magnetic field pulses, PeerJ 5 (2017), e3267.
[19] A. Golberg, M. Sack, J. Teissie, G. Pataro, U. Pliquett, G. Saulis, S. Töpfl, D. Miklavčič, E. Vorobiev, W. Frey, Energy-efficient biomass processing with pulsed electric fields for bioeconomy and sustainable development, Biotechnol. Biofuels 9 (94) (2016).
[20] S. Haberl, D. Miklavčič, G. Serša, W. Frey, B. Rubinsky, Cell membrane electroporation – part 2: the applications, IEEE Electr. Insul. Mag. 29 (1) (2013) 29–37.
[21] T. Kotnik, W. Frey, M. Sack, S. Haberl Meglič, M. Peterka, D. Miklavčič, Electroporation-based applications in biotechnology, Trends Biotechnol. 33 (2015) 480–488.
[22] L. Rems, D. Miklavčič, Tutorial: electroporation of cells in complex materials and tissue, J. Appl. Phys. 119 (2016), 201101.
[23] M. Pavlin, M. Kandušer, M. Reberšek, G. Pucihar, F.X. Hart, R. Magjarević, D. Miklavčič, Effect of cell electroporation on the conductivity of a cell suspension, Biophys. J. 88 (6) (2005) 4378–4390.
[24] T.Y. Tsong, Electroporation of cell membranes, Biophys. J. 60 (2) (1991) 297–306.
[25] M.P. Rols, J. Teissié, Electropermeabilization of mammalian cells. Quantitative analysis of the phenomenon, Biophys. J. 58 (5) (1990) 1089–1098.
[26] 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 (2–3) (1999) 233–240.
[27] D. Miklavčič, K. Beravs, D. Šemrov, M. Čemažar, F. Demšar, G. Serša, The importance of electric field distribution for effective in vivo electroporation of tissues, Biophys. J. 74 (1998) 2152–2158.
[28] M. Pavlin, N. Pavselj, D. Miklavcic, Dependence of induced transmembrane potential on cell density, arrangement, and cell position inside a cell system, IEEE Trans. Biomed. Eng. 49 (6) (2002) 605–612.
[29] G. Pucihar, T. Kotnik, J. Teissié, D. Miklavcic, Electropermeabilization of dense cell suspensions, Eur. Biophys. J. 36 (3) (2007) 173–185.
[30] J. Langus, M. Kranjc, B. Kos, M. Šuštar, D. Miklavčič, Dynamic finite-element model for efficient modelling of electric currents in electroporated tissue, Sci. Rep. 6 (26409) (2016) 2016.
[31] S. Corovic, I. Lackovic, P. Sustaric, T. Sustar, T. Rodic, D. Miklavcic, Modeling of electric field distribution in tissues during electroporation, Biomed. Eng. Online 12 (2013) 16.
[32] D. Šel, D. Cukjati, D. Batiuskaite, T. Slivnik, L.M. Mir, D. Miklavčič, Sequential finite element model of tissue electropermeabilization, IEEE Trans. Biomed. Eng. 52 (2005) 816–827.
[33] H. Akiyama, S. Katsuki, L. Redondo, M. Akiyama, A.J.M. Pemen, T. Huiskamp, F.R.J.C.M. Beckers, E.J.M. van Heesch, G.J.J. Winands, S.J. Voeten, L. Zhen, J.W.M. van Bree, S. Xiao, R. Petrella, in: H. Akiyama, R. Heller (Eds.), Pulsed Power Technology, Springer, Japan, 2017 (Bioelectrics, Chap 2, doi 10.1007/978-4-431-56095-1_2).
[34] L.M.S. Redondo, Basic concepts of high-voltage pulse generation, in: D. Miklavcic (Ed.), Handbook of Electroporation, Springer 2017, pp. 1–21, https://doi.org/10.1007/978-3-319-26779-1_209-1.
[35] E. Pirc, M. Reberšek, D. Miklavčič, Dosimetry in electroporation-based technologies and treatments, in: M.S. Markov (Ed.), Dosimetry in Bioelectromagnetic, CRC Press, Boca Raton 2017, pp. 233–268.
[36] M. Reberšek, in: D. Miklavcic (Ed.), Beyond Electroporation Pulse Parameters: From Application to Evaluation Handbook of Electroporation, Springer 2017, pp. 1–21, https://doi.org/10.1007/978-3-319-26779-1_222-1.
[37] D. Miklavčič, D. Šemrov, H. Mekid, L.M. Mir, A validated model of in vivo electric field distribution in tissues for electrochemotherapy and for DNA electrotransfer for gene therapy, Biochim. Biophys. Acta 1523 (2000) 73–83.
[38] F. Maglietti, S. Michinski, N. Olaiz, M. Castro, C. Suárez, G. Marshall, The role of pH fronts in tissue electroporation based treatments, PLoS One 8 (11) (2013), e80167https://doi.org/10.1371/journal.pone.0080167.
[39] G. Pataro, M. Falcone, G. Donsì, G. a Ferrari, Metal release from stainless steel electrodes of a PEF treatment chamber: effects of electrical parameters and food composition, Innovative Food Sci. Emerg. Technol. 21 (2014) 58–65.
[40] N. Pavšelj, D. Miklavčič, Numerical modeling in electroporation-based biomedical applications, Radiol. Oncol. 42 (2008) 159–168.
[41] L.G. Campana, B. Mali, G. Serša, S. Valpione, C.A. Giorgi, P. Strojan, D. Miklavčič, C.R. Rossi, Electrochemotherapy in non-melanoma head and neck cancers: a retrospective analysis of the treated cases, Br. J. Oral Maxillofac. Surg. 52 (2014) 957–964.
[42] O.N. Pakhomova, B.W. Gregory, V.A. Khorokhorina, A.M. Bowman, S. Xiao, A.G. Pakhomov, Electroporation-induced electrosensitization, PLoS One 6 (2) (2011), e17100https://doi.org/10.1371/journal.pone.0017100 (Launikonis B, editor).
[43] O.N. Pakhomova, B.W. Gregory, A.G. Pakhomov, Facilitation of electroporative drug uptake and cell killing by electrosensitization, J. Cell. Mol. Med. 17 (2013) 154–159.
[44] B. Zorec, S. Becker, M. Reberšek, D. Miklavčič, N. Pavšelj, Skin electroporation for transdermal drug delivery: the influence of the order of different square wave electric pulses, Int. J. Pharm. 457 (2013) 214–223.
[45] T. Blagus, B. Markelc, M. Cemazar, T. Kosjek, V. Preat, D. Miklavcic, G. Sersa, In vivo real-time monitoring system of electroporation mediated control of transdermal and topical drug delivery, J. Control. Release 172 (3) (2013) 862–871, https://doi.org/10.1016/j.jconrel.2013.09.030.
[46] A. Donate, D. Coppola, Y. Cruz, R. Heller, Evaluation of a novel non-penetrating electrode for use in DNA vaccination, PLoS One 6 (4) (2011), e19181.
[47] F. Lin, X. Shen, G. Kichaev, J.M. Mendoza, M. Yang, P. Armendi, J. Yan, G.P. Kobinger, A.J. Bello, A. Khan, K.E. Broderick, N.Y. Sardesai, Optimization of electroporation-enhanced intradermal delivery of DNA vaccine using a minimally invasive surface device, Hum. Gene Ther. 23 (3) (2012) 157–168, https://doi.org/10.1089/hgtb.2011.209.
[48] T. Kotnik, G. Pucihar, M. Rebersek, D. Miklavcic, L.M. Mir, Role of pulse shape in cell membrane electropermeabilization, Biochim. Biophys. Acta 1614 (2) (2003) 193–200.
[49] A.G. Pakhomov, I. Semenov, S. Xiao, O.N. Pakhomova, B. Gregory, K.H. Schoenbach, J.C. Ullery, H.T. Beier, S.R. Rajulapati, B.L. Ibey, Cancellation of cellular responses to nanoelectroporation by reversing the stimulus polarity, Cell. Mol. Life Sci. 71 (22) (2014) 4431–4441, https://doi.org/10.1007/s00018-014-1626-z.
[50] C.M. Valdez, R.A. Barnes Jr., C.C. Roth, E.K. Moen, G.A. Throckmorton, B.L. Ibey, Asymmetrical bipolar nanosecond electric pulse widths modify bipolar cancellation, Sci. Rep. 7 (1) (2017) 16372, https://doi.org/10.1038/s41598-017-16142-6.
[51] C. Muratori, M. Casciola, O. Pakhomova, in: D. Miklavcic (Ed.), Electric Pulse Repetition Rate: Sensitization and Desensitization Handbook of Electroporation, Springer 2017, pp. 1–16, https://doi.org/10.1007/978-3-319-26779-1_23-1.
[52] G. Pucihar, L.M. Mir, D. Miklavcic, The effect of pulse repetition frequency on the uptake into electropermeabilized cells in vitro with possible applications in electrochemotherapy, Bioelectrochemistry 57 (2) (2002) 167–172.
[53] P.A. Garcia, R.V. Davalos, D. Miklavcic, A numerical investigation of the electric and thermal cell kill distributions in electroporation-based therapies in tissue, PLoS One 9 (8) (2014), e103083https://doi.org/10.1371/journal.pone.0103083.
[54] C. Faurie, E. Phez, M. Golzio, C. Vossen, J.C. Lesbordes, C. Delteil, J. Teissié, M.P. Rols, Effect of electric field vectoriality on electrically mediated gene delivery in mammalian cells, Biochim. Biophys. Acta 1665 (1–2) (2004) 92–100.
[55] P. Hojman, H. Gissel, F.M. Andre, C. Cournil-Henrionnet, J. Eriksen, J. Gehl, L.M. Mir, Physiological effects of high- and low-voltage pulse combinations for gene electrotransfer in muscle, Hum. Gene Ther. 19 (11) (2008) 1249–1260, https://doi.org/10.1089/hum.2008.059.
[56] Q. Hu, R.P. Joshi, Comparative evaluation of transmembrane ion transport due to monopolar and bipolar nanosecond, high-intensity electroporation pulses based on full three-dimensional analyses, J. Appl. Phys. 122 (034701) (2017)https://doi.org/10.1063/1.4994310.

[57] V. Todorović, U. Kamenšek, G. Serša, M. Čemažar, Changing electrode orientation, but not pulse polarity, increases the efficacy of gene electrotransfer to tumors *in vivo*, Bioelectrochemistry (2014) 1567–5394, https://doi.org/10.1016/j.bioelechem.2013.12.002 ([Print ed.], str. [1-9]).
[58] L. Chicaybam, A.L. Sodre, B.A. Curzio, M.H. Bonamino, An efficient low cost method for gene transfer to T lymphocytes, PLoS One 8 (3) (2013), e60298https://doi.org/10.1371/journal.pone.0060298.
[59] D.C. Sweeney, M. Reberšek, J. Dermol, L. Rems, D. Miklavčič, R.V. Davalos, Quantification of cell membrane permeability induced by monopolar and high-frequency bipolar bursts of electrical pulses, Biochim. Biophys. Acta 1858 (11) (2016) 2689–2698, https://doi.org/10.1016/j.bbamem.2016.06.024.
[60] M. Essone Mezeme, G. Pucihar, M. Pavlin, C. Brosseau, D. Miklavčič, A numerical analysis of multicellular environment for modeling tissue electroporation, Appl. Phys. Lett. 100 (2012), 143701.
[61] R. Susil, D. Šemrov, D. Miklavčič, Electric field induced transmembrane potential depends on cell density and organization, Electro- Magnetobiol. 17 (1998) 391–399.
[62] T. Dolinsek, G. Sersa, L. Prosen, M. Bosnjak, M. Stimac, U. Razborsek, M. Cemazar, Electrotransfer of plasmid DNA encoding an anti-mouse endoglin (CD105) shRNA to B16 melanoma tumors with low and high metastatic potential results in pronounced anti-tumor effects, Cancers (Basel) 8 (1) (2015)https://doi.org/10.3390/cancers8010003 (pii: E3).
[63] L. Heller, V. Todorovic, M. Cemazar, Electrotransfer of single-stranded or double-stranded DNA induces complete regression of palpable B16.F10 mouse melanomas, Cancer Gene Ther. 20 (12) (2013) 695–700, https://doi.org/10.1038/cgt.2013.71.
[64] C.J. Mann, X.M. Anguela, J. Montane, M. Obach, C. Roca, A. Ruzo, P. Otaegui, F. Mir LM Bosch, Molecular signature of the immune and tissue response to non-coding plasmid DNA in skeletal muscle after electrotransfer, Gene Ther. 19 (2012) 1177–1186, https://doi.org/10.1038/gt.2011.198.
[65] K. Znidar, M. Bosnjak, M. Cemazar, L.C. Heller, Cytosolic DNA sensor upregulation accompanies DNA electrotransfer in B16.F10 melanoma cells, Mol. Ther.–Nucleic Acids 5 (2016)https://doi.org/10.1038/mtna.2016.34 (ISSN 2162-2531, http://www.nature.com/mtna/journal/v5/n6/full/mtna201634a.html).
[66] E. Bellard, B. Markelc, S. Pelofy, F. Le Guerroué, G. Sersa, J. Teissié, M. Cemazar, M. Golzio, Intravital microscopy at the single vessel level brings new insights of vascular modification mechanisms induced by electropermeabilization, J. Control. Release 163 (3) (2012) 396–403, https://doi.org/10.1016/j.jconrel.2012.09.010.
[67] M. De Robertis, U. Lampreht Tratar, M. Cemazar, E. Signori, in: D. Miklavcic (Ed.), Predictable Animal Models for Translational Electroporation-Based Cancer Immunotherapy Studies Handbook of Electroporation, Springer 2016, pp. 1–21, https://doi.org/10.1007/978-3-319-26779-1_50-1.
[68] F.M. André, C. Cournil-Henrionnet, D. Vernerey, P. Opolon, L.M. Mir, Variability of naked DNA expression after direct local injection: the influence of the injection speed, Gene Ther. 13 (23) (2006) 1619–1627.
[69] T. Jarm, M. Cemazar, D. Miklavcic, G. Sersa, Blood flow-modifying and vascular-disrupting effects of electroporation and electrochemotherapy – implications in treatment of melanoma metastases, Expert. Rev. Anticancer. Ther. 10 (5) (2010) 729–746.
[70] M. Čemažar, R. Milačič, D. Miklavčič, V. Dolžan, G. Serša, Intratumoral cisplatin administration in electrochemotherapy: antitumor effectiveness, sequence dependence and platinum content, Anti-Cancer Drugs 9 (1998) 525–530.
[71] S. Corović, A. Zupanic, S. Kranjc, B. Al Sakere, A. Leroy-Willig, L.M. Mir, D. Miklavcic, The influence of skeletal muscle anisotropy on electroporation: in vivo study and numerical modeling, Med. Biol. Eng. Comput. 48 (7) (2010) 637–648.
[72] S. Corovic, B. Al Sakere, V. Haddad, D. Miklavcic, L.M. Mir, Importance of contact surface between electrodes and treated tissue in electrochemotherapy, Technol. Cancer Res. Treat. 7 (5) (2008) 393–400.
[73] A. Ivorra, B. Al-Sakere, B. Rubinsky, L.M. Mir, In vivo electrical conductivity measurements during and after tumor electroporation: conductivity changes reflect the treatment outcome, Phys. Med. Biol. 54 (19) (2009) 5949–5963, https://doi.org/10.1088/0031-9155/54/19/019.
[74] N. Abou-Madi, Anesthesia and analgesia of small mammals, in: R.D. Gleed, J.W. Ludders (Eds.), Recent Advances in Anesthesia and Analgesia: Companion Animals, International Veterinary Information Service, Ithaca, NY, 2006 (online).
[75] D.F. Kohn, S.K. Wixson, W.J. White, G.J. Benson, Anesthesia and Analgesia in Laboratory Animals, Academic Press, New York, 1997.
[76] R.I. Mazze, A.J. Wilson, S.A. Rice, J.M. Baden, Fetal development in mice exposed to isoflurane, Teratology 32 (1985) 339–345.
[77] Y. Miwa, K.K. Sladky, Small Mammals: Common Surgical Procedures of Rodents, Ferrets, Hedgehogs, and Sugar Gliders, Vet. Clin. North Am. Exot. Anim. Pract. 19 (1) (2016) 205–244, https://doi.org/10.1016/j.cvex.2015.09.001.
[78] D. Cressey, News Nature, 2011https://doi.org/10.1038/news.2011.391 (Published online 29 June 2011).

Cemazar M. received her PhD in Basic Medical Sciences from the Medical Faculty, University of Ljubljana in 1998. She works at the Department of Experimental Oncology, Institute of Oncology Ljubljana and teaches at the Faculty of Health Sciences, University of Primorska, Slovenia. Her main research interests are in the field of gene electrotransfer employing plasmid DNA encoding different immunomodulatory and antiangiogenic therapeutic genes. In 2006 she received the Award of the Republic of Slovenia for important achievements in scientific research and development in the field of experimental oncology.

Sersa G. graduated from the Biotechnical Faculty at the University of Ljubljana in 1978, where he is currently a professor of molecular biology. He is employed at the Institute of Oncology in Ljubljana as a head of the Department of Experimental Oncology. His specific field of interest is the effect of electric field on tumor cells and tumors as drug and gene delivery system in different therapeutic approaches. Beside experimental work, he is actively involved in the education of undergraduate and postgraduate students at the University of Ljubljana.

Frey W. received the Diploma degree in high voltage technology and the Ph.D. degree in laser triggering of rail gap switches from the University of Karlsruhe, Karlsruhe, Germany, in 1989 and 1996, respectively. He was an Assistant Professor with the High Voltage Institute, the University of Karlsruhe, Germany, on new pulse forming concepts, high voltage test engineering, and gas insulated spark gaps. In 1997, he joined the Pulsed Power Group of the former Research Center of Karlsruhe, Karlsruhe, Germany. He is currently with Karlsruhe Institute of Technology (KIT). He started with surface coating by pulsed electron beam ablation, conducted research and engineering on electrodynamic fragmentation for material processing and switched to pulsed electric field effects on biological matter in 2001. He focused on application-oriented research on pulsed electric field treatment for bacterial decontamination and cell ingredient extraction and on basic diagnostics for ns-timescale membrane-voltage-dynamics measurement. Since 2006, he has been a Team Leader in Bioelectrics with the Institute for Pulsed Power and Microwave Technology, KIT, Campus North. His current research interests include pulsed electric field processing of microalgae for cell component extraction.

Miklavcic D. was born in 1963. He received a Ph.D. in Electrical Engineering from the University of Ljubljana, Slovenia in 1993. He is currently a full professor, head of the Laboratory of Biocybernetics, and head of the Department of Biomedical Engineering at the Faculty of Electrical Engineering of the University of Ljubljana. During the last few years his research has been focused on electroporation-based gene transfer and drug delivery, development of electronic hardware, and numerical modeling of biological processes.

Teissié J. born in 1947, got a degree in engineering from ESPCI (Paris, 1970), a PhD in macromolecular chemistry (Université Paris VI, 1973), a DSc in Biophysics (Université Toulouse, 1979). Hired by the CNRS in 1973, post doc fellow at the Johns Hopkins University (School of Medicine) (1979–81). He is DRCE CNRS Emeritus. Field of expertise - bioelectrochemical aspects of membrane biophysics. His research is a synergy between development of new hypothesis and concepts and experimental validation through designs of new technologies. His contribution to electropermeabilization and related phenomena is considered as a major one worldwide. Author of more than 200 papers and recipient of several scientific prices. More recently he was the recipient of the F Reidy Prize in 2012. He was elected as an honorary senator of the University of Ljubljana in 2015 and as a fellow of the AIMBE in 2017.