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Effects of high voltage nanosecond electric pulses on eucaryotic cells (*in vitro*): A systematic review

Tina Batista Napotnik [a], Matej Reberšek [a], P. Thomas Vernier [b], Barbara Mali [a], Damijan Miklavčič [a,*]

[a] University of Ljubljana, Faculty of Electrical Engineering, Department of Biomedical Engineering, Laboratory of Biocybernetics, Tržaška 25, 1000 Ljubljana, Slovenia
[b] Frank Reidy Research Center for Bioelectrics, Old Dominion University, Norfolk 4211, Monarch Way, VA, USA

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ABSTRACT

For this systematic review, 203 published reports on effects of electroporation using nanosecond high-voltage electric pulses (nsEP) on eukaryotic cells (human, animal, plant) *in vitro* were analyzed. A field synopsis summarizes current published data in the field with respect to publication year, cell types, exposure configuration, and pulse duration. Published data were analyzed for effects observed in eight main target areas (plasma membrane, intracellular, apoptosis, calcium level and distribution, survival, nucleus, mitochondria, stress) and an additional 107 detailed outcomes. We statistically analyzed effects of nsEP with respect to three pulse duration groups: A: 1–10 ns, B: 11–100 ns and C: 101–999 ns. The analysis confirmed that the plasma membrane is more affected with longer pulses than with short pulses, seen best in uptake of dye molecules after applying single pulses. Additionally, we have reviewed measurements of nsEP and evaluations of the electric fields to which cells were exposed in these reports, and we provide recommendations for assessing nanosecond pulsed electric field effects in electroporation studies.

Contents

Abbreviations: nsEP, nanosecond electric pulses; FWHM, full width at half maximum; PI, propidium iodide; TB, trypan blue; EtH, ethidium homodimer; YOPRO, YO-PRO®-1 iodide; FITC, fluorescein isothiocyanate; GFP, green fluorescent protein; PEG, polyethylene glycol; PM, plasma membrane; SEM, scanning electron microscope; TDDS, time domain dielectric spectroscopy; TDR, time domain reflectometry; ER, endoplasmic reticulum; PARP, poly (ADP-ribose) polymerase; PS, phosphatidylserine; Tl^{+}, thallium ion; Ca^{2+}, calcium ion; BAPTA, 1,2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid); EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid; ROS, reactive oxygen species; MAPK, mitogen-activated protein kinase; AMPK, AMP-activated protein kinase; cyt c, cytochrome c.

* Corresponding author at: Faculty of Electrical Engineering, Tržaška 25, 1000 Ljubljana, Slovenia.
E-mail address: damijan.miklavcic@fe.uni-lj.si (D. Miklavčič).

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1. Introduction

Exposure of biological materials (cell suspensions, tissues) to high voltage electric pulses provokes a phenomenon that is nowadays termed electroporation: cell membranes become more permeable to molecules that otherwise cannot cross them [1–3]. Although not completely understood, electroporation has been widely studied and used as a basis for applications in medicine and biotechnology such as electrochemotherapy [4,5], gene electrotransfer [6], tissue ablation [7,8], extraction of various compounds [9,10], and microbial inactivation in food preservation [11].

Early theoretical predictions [12,13] and experiments [12,14,15] showed that shorter electric pulses of nanosecond duration (nsEP stands for nanosecond electric pulses; often called "nanosecond pulsed electric field" or nsPEF) have more profound effects on the cell interior than longer pulses of micro- and millisecond duration, and thus nsEP emerged as a promising tool for intracellular manipulation without any chemical intervention. With the development of new pulse generators that produced ultrashort pulses of very high electric fields of several tens of MV/m [16], researchers were able to show effects on cell organelles. In the last 15 years we have gained considerable knowledge about how nanosecond electric pulses affect cells: they affect cell organelles [15,17,18], increase intracellular calcium [19–21], and provoke apoptosis [22–24] and stress responses [25].

Researchers gradually discovered that the plasma membrane is also affected. The pores produced by nsEP are small, of nanometer scale, and are thus sometimes called "nanopores" [26–28]. Cells exposed to nsEP exhibit membrane permeability to both propidium (PI) and trypan blue (TB), classical indicators of membrane permeabilization, but detecting influx of these dyes and other small molecules after nanosecond pulse exposure requires methods with greater sensitivity than those used for longer pulses.

However, researchers using different cells, pulse parameters, exposure configuration and detection methods have described results that are often contradictory. Therefore, the first aim of this study was to review published results in a systematic and comprehensive way. Secondly, we used statistical analysis of published results to determine whether nsEP of different durations affect cells differently.

We classified nsEP into three distinct categories: A: 1–10 ns, B: 11–100 ns and C: 101–999 ns. With the use of nanosecond pulses, as pulses shorten, more intracellular effects and less effects on plasma membrane can be expected [15,19,29,30]. The first category (A) includes very short pulses, 1–10 ns, with rise times of a few ns (mostly shorter than the electrolyte relaxation time). In this regime the dielectric properties of the membrane and the intracellular and extracellular media dominate pulsed electric field effects on membranes, and the Maxwell–Wagner polarization of the membrane by migration of mobile charges is less important than it is for longer pulses [31]. Moreover, proportionally greater effects are expected on intracellular membranes with 1–10 ns pulses than with longer pulses [32,33]. In the second category (B), the pulse duration is less than the charging time of the plasma membrane [15,34,35]. The third category (C) includes nsEP with durations longer than the plasma membrane charging time.

The main focus of this review was the role of pulse duration in reported effects of nsEP on biological cells. With the use of statistical methods we analyzed all published data of experimental studies that examined effects of nsEP on eukaryotic human, animal, and plant cells *in vitro*. We tested several hypotheses. The main two were the following: 1. the occurrence of changes in the plasma membrane significantly depends on nsEP pulse duration, and 2. the occurrence of intracellular effects significantly depend on nsEP pulse duration. We hypothesized that PM effects are greater with longer pulses and intracellular effects are greater with shorter pulses. We also tested similar hypotheses for a number of effects of nsEP on cells *in vitro* that are reported in the literature.

Our second focus was the evaluation of the nanosecond pulsed electric fields used in experimental studies of electroporation (electropermeabilization). During the electroporation process biological cells are exposed to pulsed electric fields with specific electrical parameters, namely amplitude, duration, shape, number of pulses and pulse repetition rate. The duration of the pulse is usually specified as the full width at half maximum (FWHM) and a description of the pulse shape is usually enhanced with rise and fall times of the pulse [36]. In order to exactly specify the experimental method and thus to enable the reproduction of experiments under the same conditions, researchers should exactly determine and describe these electrical parameters. Some electrical parameters such as number of pulses and pulse repetition rate are relatively easy to state. Other electrical parameters are more difficult to determine, because it is currently not possible to measure the time course and distribution of the nanosecond electric field within the exposure configuration during the delivery. Our hypothesis was that in the existing literature on the electroporation of biological cells by nsEP there is not enough emphasis on the determination and description of the electric field to which biological cells are exposed or they are not described in adequate detail. By analyzing the data from the published reports, we compiled a list of recommendations for the evaluation of nanosecond electric field to which cells are exposed in electroporation studies.

2. Methods

2.1. Eligibility criteria

In our systematic review, we included reports of experimental studies in which eukaryotic human, animal, and plant cells were exposed to electric pulses of nanosecond duration *in vitro*.

2.2. Search strategy and study selection

For the purpose of our review, a systematic search through eight online bibliographic databases (Science Direct, IEEE Xplore, SpringerLink, Web of Science, HighWire Press, Compendex, IngentaConnect, PubMed) was performed by TBN on August 9, 2013, by employing keywords with Boolean operators ("nanosecond electric pulses" OR "nanosecond pulsed electric field") AND "cells". In most cases the search was confined to the years 1990–2013. We included scientific articles and conference reports (also reports accepted for publication and published online) written in English, and excluded books, abstracts, and unpublished data. Briefly, records clearly not related to the theme (from other research fields such as physics and chemistry) were excluded on the