Biological effects of ultrashort pulsed electric fields

J.F. Kolb[1,2], S.J. Beebe[1], M. Stacey[1], R.P. Joshi[2], S. Xiao[1,2], K.H. Schoenbach[1]
[1]Frank Reidy Research Center for Bioelectrics, Old Dominion University, Norfolk, Virginia, USA
[2]Department of Electrical and Computer Engineering, Old Dominion University, Norfolk, Virginia, USA
e-mail: jkolb@odu.edu

Résumé

Exposures of cells to pulsed electric fields of ultrashort duration (nanoseconds) and high field strengths (tens of kilovolts per centimeter) can alter cell morphologies and functions. On this basis, applications can be devised for bacterial decontamination, wound healing, and cancer treatment.

Introduction

Exposures of cells in electric fields are known for the various responses, which depend on the specific parameters of the applied electric fields, in particular field strength and duration. Electromagnetic radiations with frequencies of hundreds of kilohertz (i.e. radio frequencies) up to some gigahertz (microwaves) are becoming main stream therapies to induce hyperthermia in tumors or to ablate tissues. Results are achieved by increasing the temperature at the target site to much higher levels than are tolerable for mammalian cells. Biological responses for power levels that do not lead to significant heating (or even a measureable increase in temperature) are discussed controversial, and are mostly associated with hazards from cell phone use and risks of ambient extremely low frequency electromagnetic fields (ELF).

Conversely, pulsed electric fields are used to deliberately induce non-thermal responses. Pulses with durations of microseconds to milliseconds are used to open cell membranes for the transfer of otherwise membrane impermeable large molecules, such as drugs or DNA [1]. Whereas the longer pulses more or less exclusively affect the outer cell membranes, nanosecond pulsed electric fields (nsPEFs), can in addition effectively reach organelles and subcellular structures [2,3]. The subsequent observed biological response depends on the specific cell (cell type and environment) and details of the applied stimulus (pulse duration, pulse amplitude, pulse number and pulse repetition rate). Consequently, different exposure schemes have been developed for various applications. The induction of subcellular effects can be used for the killing of bacteria or just the transient inactivation, i.e. stunning of microorganisms [4]. As trigger for biochemical cascades, nsPEFs can cause functional changes, and for example initiate the aggregation of platelets [5]. Another most promising response, is the induction of apoptosis, which holds the promise of new tumor therapies [6,7].

Interaction of nanosecond pulse electric field exposures with cells

The immediate responses of mammalian cells to the exposure in a pulsed electric field can be attributed to the charging of the plasma membrane. When the transmembrane voltage reaches a cell-line specific threshold value, which is generally on the order of 1 V, pores form in the membrane that allow ions and larger molecules like DNA to cross into and out of the cell. The mechanism is known as electroporation, refering to the application of pulses of several microseconds to milliseconds. If the duration of the pulse is shorter than the charging time of the outer membrane and pulse amplitudes are high, also membranes of internal organelles will be affected in addition to the plasma membrane. Depending on pulse parameters, the results of this stimulation vary, from a mere disturbance of cell function to the induction of apoptosis. To investigate the conditions for the induction of all these physiological reponses, we recorded the changes of the transmembrane potential during the application of a nanosecond pulsed electric field in real time, i.e., with a temporal resolution of 5 ns – short compared to the duration of the pulse. The results show that the intense fast-rising pulses lead to an immediate change of about 1 V at the anodic side of the cell. The subsequent development depends on the pulse amplitude. Once a value of 1.4-1.6 V is reached, decreasing transmembrane changes indicate the formation of pores, as was anticipated by modeling efforts. The observed changes in transmembrane potentials correspond to the increase of intracellular calcium levels several milliseconds after exposure for the same pulse conditions. Only for the highest electric field strength is this response dependent on the presence of extracellular calcium. This suggests that, except for this last case, calcium is released mostly from the cell's internal stores. The fast rise in calcium in about 2-4 ms again suggests the outflow through pores. Ultimately, a better understanding of these ultrafast, non-physiological responses will allow us to manipulate cells to obtain a specific desired effect.

Environmental and medical applications of nanosecond pulsed electric field exposures

Pulses in the range of 0.1-100 kV/cm amplitude have been shown effective in biofouling prevention by either causing cell lysis or transient inactivation. The electrical energy that is required for lysis of bacteria can be reduced by decreasing the duration of the applied pulses. Lysis of *E.coli* was achieved with duration as short as 60 ns. More complex organism, such as brine shrimp (*artemia salina*) responded to pulsed electric fields with a temporary inactivation, i.e. were being stunned. For a more than 2 minute inactivation, the energy that had to be delivered by a 60-ns pulse corresponds to an energy density of 2 J/cm^3. Interestingly, stunning could be achieved with a ten times lower energy, for a 5-µs. pulse. This might indicate that a reversible disturbance of neurotransmitter signals between cells has significant contribution to the inactivation.

Currently the most prominent application of nanosecond pulsed electric fields is the possible cure for certain types of cancer. In vitro experiments have shown that exposures can lead to the expression of caspases that are known to lead to apoptosis. Applied to tumors in vivo, in addition the disruption of angiogenesis has been observed. Other mechanisms and their synergy seems also possible. The first survival study in mice demonstrated the total remission of B16 murine melanoma tumors and the long term survival of the animals after multiple treatments with 300-ns pulses. The success was recently repeated for HEP 1-6 murine liver cancers treated with 100-ns pulses.

Exposures of solid tumors so far require subcutaneous needle electrodes, which can possible be developed into laparoscopic procedures. Alternatively reducing the pulse duration into the sub-nanosecond range enables driving wideband radiation sources, which can deliver high electric fields deep into the body. In pursuit of this goal, we investigated the effect of 200-ps pulses and concurrently the radiation characteristics of prolate-spheroidal antennas, when excited by such a pulse [8].

References

[1] S.B. Dev, D.P. Rabussay, G. Widera, G.A. Hofmann, IEEE Trans. Plasma Sci. **28** (2000) 206.

[2] K.H. Schoenbach, R.P. Joshi, J.F. Kolb, N.Y. Chen, M. Stacey, P.F. Blackmore, E.S. Buescher, S.J. Beebe, *Ultrashort electrical pulses open a new gateway into biological cells*, IEEE 92 (2004) 1122.

[3] T.R. Gowrishankar, A.T. Esser, Z. Vasilkoski, K.C. Smith, J.C. Weaver, *Microdosimetry for conventional and supra-electroporation in cells with organelles*, BBRC 341 (2006) 1266.

[4] K.H. Schoenbach, F.E. Peterkin, R.W. Alden III, S.J. Beebe, IEEE Trans. Plasma Sci. **25** (1997) 284.

[5] K.H. Schoenbach, B.Hargrave, R.P. Joshi, J.F. Kolb, R. Nuccitelli, C. Osgood, A. Pakhomov, M. Stacey, R.J. Swanson, J.A White, S. Xiao, J. Zhang, S.J. Beebe, P.F. Blackmore, E.S. Buescher, IEEE Trans. Dielec. Electr. Insul. **14** (2007) 1088.

[6] R. Nuccitelli, X. Chen, A.G. Pakhomov, W.H. Baldwin, S. Sheikh, J.L. Pomicter, W. Ren, C. Osgood, R.J. Swanson, J.F. Kolb, S.J. Beebe, K.H. Schoenbach, Int. J. Cancer. **125** (2009) 438.

[7] X. Chen, J.F. Kolb, R.J. Swanson, K.H. Schoenbach, S.J. Beebe, Pigment Cell Melanoma Res. **23** (2010) 554.

[8] K.H. Schoenbach, S. Xiao, R.P. Joshi, T. Camp, T. Heeren, J.F. Kolb, S.J. Beebe, IEEE Trans. Plasma. Sci. **36** (2008) 414.