Published in final edited form as:

Med Biol Eng Comput. 2017 July ; 55(7): 1063–1072. doi:10.1007/s11517-016-1516-7.

A Subnanosecond Electric Pulse Exposure System for Biological Cells

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Abstract

An exposure system adapted for use on a microscope stage was constructed for studying the effects of high electric field, subnanosecond pulses on biological cells. The system has a bandpass of 3 GHz and is capable of delivering high voltage electric pulses (6.2 kV) to the electrodes, which are two tungsten rods (100 μm in diameter) in parallel with a gap distance of 170 μm . Electric pulses are delivered to the electrodes through a π -network, which serves as an attenuator as well as an impedance matching unit to absorb the reflection at the electrodes. By minimizing the inductance of the pulse delivery system, it was possible to generate electric fields of up to 200 kV/cm with a pulse duration of 500 ps at the surface of the cover slip under the microscope. The electric field at the cover slip was found to be homogenous over an area of 50 to 70 μm . Within this area, neuroblastoma cells placed on the cover slip were studied with respect to membrane potential changes caused by subnanosecond pulses. This allowed us, for the first time, to demonstrate depolarization of the cell membrane potential.

Keywords

subnanosecond pulses; exposure system; transmembrane potential

1. Introduction

Electrical pulses, long compared to the membrane charging time constant, which is defined by the capacitance of the membrane and the resistances of the medium and the cytoplasm, increase the voltage across the cell membrane. Consequently the electric field in a 5 nm thick membrane can become high [1, 2]. Electroporation is anticipated once it reaches 400 kV/cm to 2.8 MV/cm corresponding to voltages of 200 mV to 1.4 V [3–6]. The charging time constant for cell membranes with diameters of 10 μm is on the order of 100 ns as estimated from a Cole's single-shell model. It is larger for larger cells, and also decreases with increasing conductivities of the cytoplasm and medium [7]. Pulses that are short compared to the membrane charging time constant allow the direct interaction of pulse electric field with the nucleus or other intracellular organelles, in addition to charging of the

membrane [8]. It was originally assumed that nanosecond pulses can mainly induce intracellular effects. However, later experimental results showed that such intracellular effects are accompanied by the generation of nanopores in the cell membrane [9].

As the duration of the pulses is reduced into the subnanosecond range, capacitive properties of cytoplasm and external medium in addition to the conductive properties must be taken into account [10]. Applying subnanosecond pulses to cells still causes an increase in membrane voltage, but increasingly more due to differences in permittivity between the cell membrane (low permittivity), cytoplasm and medium (high permittivity). In addition, the dielectric relaxation of the cell membrane plays an increasing role in the electrical power deposition into the cell membrane. Modeling this effect in the frequency range has shown that the power deposition into the membrane increases by an order of magnitude for frequencies above about 100 MHz (corresponding to pulse duration on the order of a nanosecond and below) [11]. Studying the effects of such short pulses (and correspondingly high frequencies) opens up a new field of electric field-cell interactions, quite different from conventional electroporation effects observed for pulses in the nanosecond to millisecond range. But unlike nanosecond pulses and longer electric pulses for which systematic studies have been conducted, the prime question of whether large amplitude subnanosecond electric pulses cause intracellular or plasma membrane effects remains unanswered.

A number of studies of biological effects caused by lower amplitude subnanosecond pulses were carried out in rats in the 1990s, when Electromagnetic Pulses (EMP) were assessed for their potential hazard. The results consistently showed no detectable effects on physiological conditions, including blood pressure, heart rate etc [12–15]. It was later pointed out that the electric fields in these experiments were much lower than that required to cause measurable responses [16]. Recent studies on B-16 melanoma, liver cancer cells and HeLa cells using 800 ps [17–19] and 200 ps [20] pulses also showed that very high electric fields (from 20 kV/cm to 1000 kV/cm) were needed to cause effects such as the uptake of Trypan Blue or, as a downstream effect, apoptosis. A recent paper [21] showed that a single subnanosecond pulse (500 ps) can open voltage gated calcium channels, with a required electric field of 190 kV/cm. Besides these reports, experimental studies of subnanosecond pulse effects have not been reported. The reason for the scarcity of biological studies with intense subnanosecond pulses is mainly due to the missing availability of reliable subnanosecond exposure systems for such biological studies.

Conventional exposure systems for studying the biological effects of short pulses are mostly based on a Transverse Electromagnetic (TEM) Cell [22], in which biological cells are contained in a flask. The average electric field for cells is thus rather low as the physical size of the systems is large. One can increase the electric field by reducing the size of the exposure system and loading the wave propagation structures directly with a medium containing cells [23]. However, the range of electric fields applied to biological cells has not reported. In this paper, we report the design and construction of a high voltage, subnanosecond pulse exposure system, which allows us to generate electric fields of up to 200 kV/cm. Unlike a TEM cell, which operates in the free space and only permits an electric field intensity not exceeding air breakdown (approximately 30 kV/cm for direct voltages), this exposure system allows much higher electric fields to be delivered to cells as the

electrodes are entirely submerged in a water-based cell medium. The problem of air breakdown due to high voltage is therefore eliminated.

With this exposure system we already have shown that a single subnanosecond pulse can cause calcium influx [21]. Here, we report for the first time studies on the effect of intense subnanosecond pulses on the membrane potential. It could be shown that a single subnanosecond pulse causes a considerable increase in transmembrane potential (depolarization). This was confirmed through both patch clamp and ratiometric fluorescence imaging. As membrane depolarization can lead to the opening of voltage gated channels or firing of action potentials, the ability of modifying membrane potential in cells by subnanosecond pulses can be useful for noninvasive antenna-based stimulation of tissues or organs.

2. Design and Construction of Pulse Exposure System

The complete setup of the exposure system can be seen in [21]. Subnanosecond electrical pulses (approximately 320 ps for Full Width Half Maximum) were generated by a FID pulse generator (FPG 20-1 PM Germany). The pulses were sent to a π network in order to absorb the downstream reflections before they were delivered to the electrodes, which can be positioned with a precision of a few μm by a micromanipulator above a cover slip under a microscope. The cells on the cover slip, positioned in the gap between the electrodes, were exposed to the field from the electrodes.

π Network

When an electric pulse is delivered to the electrodes, it is reflected partially due to an impedance mismatch at the breakout of the coaxial cable. Another reflection occurs at the end of the electrodes. The purpose of the π resistor network, placed between the pulsed power source and the electrodes, is to absorb these reflections.

A high performance resistor network in general can provide a 50 Ω termination in both input and output ports when 50 Ω coaxial cables are used for connection, but the output signal will inevitably be attenuated. A good example is a 50 Ω , 20 dB attenuator, which has a T-type configuration (40 Ω , 10 Ω , 40 Ω). For our exposure system, a π network that has three equivalent resistances: 180 Ω , 64 Ω and 180 Ω , was selected. The network acted as a 60 Ω absorber for pulses propagating in the 50 Ω coaxial cable. The attenuation for such an ideal network is 8 dB and the return loss is -18 dB.

The inductance of the pulse delivery system and the stray capacitances play an important role in shaping the pulse delivered to the load for the extremely short pulses we have used (320 ps at 50% amplitude). When constructing a resistive network for the passage of subnanosecond pulses, the main challenge thus lies in minimizing stray parameters, such as the insertion inductance and shunt capacitance. In general, placing resistors in a coaxial setting can be a good start. If the electric loop length of the resistive circuit can be restricted to the same range as the diameter of the coaxial cable being used (~1 cm for RG217, e.g.), the inductance is less than a few nH, which means that pulses with a rise time of one hundred picoseconds or less will be able to pass without distortion. For example, in a 50 Ω

cable, if a loop inductance is 5 nH, the L/Z time is 100 ps. Besides reducing the loop length, the shunt capacitance should be reduced. Even if it is not, a compensating capacitance may be added to improve the high frequency performance [24]. Furthermore, the resistors placed in the coaxial setting should cause only a minimum distortion of the radial electric field of the coaxial line. Otherwise, additional return loss would be generated, a consequence that is especially pronounced at high frequencies. Satisfying the conditions for minimum distortion therefore requires a very compact arrangement of the resistors in the π network.

In our design, the serial and parallel combination of the resistors form three equivalent resistances in the π network: 180 Ω , 64 Ω and 180 Ω , as shown in Fig. 1. They are low inductance carbon composition resistors (TDK) housed in an aluminum hollow cylinder with the same diameter as the insulation layer of an RG217 cable (9.77 mm). The overall length of the cylinder is 2 cm. The resistors were configured in a symmetrical layout, so that the resistance viewed from the left port is identical to that viewed from the right port. To increase the withstand voltage of the network, silicon glue was filled among the resistors to prevent any high-voltage flashover.

To test the π network, we first used a network analyzer (Keysight, N9926) to measure its reflection coefficient, S11 (shown in Fig. 2), a parameter that describes how much of an electromagnetic wave is reflected by impedance discontinuities. Up to 3GHz, the reflection coefficient is approximately -17 dB, close to its design value of -18dB. Beyond 3 GHz, the reflection loss becomes greater. Using a Gaussian waveform as an example, the π network can relay a pulse of 270 ps without distortion. After characterizing the reflection coefficient in the frequency domain, we used the FID pulse generator as the source and a 20X attenuator (Barth 142-NMFP-26) as the load (Fig. 3a) to test the time-domain performance of the π network. Further, we inserted an in-line V-dot probe (VDC-1-012, Farr Research) midway between the π network and the Barth attenuator to monitor the voltage. In total, we had three signals: 1) the first was from the voltage probe installed in the FID pulser; 2) the second was from the Barth probe (also serves as an attenuator) and 3) the third was from the Farr V-dot probe.

The results of testing are shown in Fig. 3b. For easy comparison, the voltage amplitudes were normalized to unity. The three waveforms are almost identical in the first 1.2 ns and each shows a pulse width of 300 ps (FWHM). However, both FID probe and Farr probe show a voltage reversal greater than 25%, whereas the Barth probe shows negative swing of less than 25%. In terms of the absolute voltage, the FID probe and Barth probe show the correct values after taking into account attenuation ratio, but the Farr probe shows a value that is higher than the predicted value. Because we didn't have technical details of the Farr probe, it was beyond our capability to analyze the cause of such discrepancy. As such, we chose to rely on the FID probe and Barth probe to obtain the magnitude. The maximum operable voltage for the π network was found to be 16 kV, which corresponds to an output of 6.2 kV.

Electrodes

Two tungsten rods (diameter: 100 μm , gap distance: 170 μm) acting as electrodes were connected to the breakout of a coaxial cable (RG316). The connection was secured by

applying epoxy glue, which also insulated the electrodes and prevented electrical breakdown (Fig. 4). There was no insulation on the other side of the tungsten rods over a distance of 3.5 mm for submerging in the cell medium. The coaxial cable with the electrodes was anchored on a brass rod and mounted on a micromanipulator for precise positioning above a cover slip with attached cells [21]. The angle of the electrodes relative to the cover slip was 45°. The intact side of the coaxial cable with the standard connector was connected to the π network.

3. Simulation of Electric Field Distribution in the Exposure Region

In the experiment, the amplitude of the subnanosecond pulse in the coaxial cable exiting from the π network was kept constant at 6.2 kV (the maximum value in the experiment). However, this was not the actual voltage across the electrodes as there were reflections due to the impedance discontinuities at the breakout of the coaxial cable (Position 1, 2 in Fig. 4) and the change in insulation between tungsten electrodes from epoxy to a water medium (position 3 in Fig. 4). The actual electric field at the surface of a cover slip with attached cells was simulated by a 3-D time-domain electromagnetic solver, CST microwave studio, taking these reflections into account. In the simulation, instead of using the real waveform, we applied a Gaussian pulse (1V, corresponding frequency 2.5 GHz) to the input port of the coaxial cable, which has the same 300 ps pulse width (FWHM) as in the experiment. The cell medium was water-based and so its electrical property can be described by a complex permittivity formula:

$$\varepsilon_r = \varepsilon_0 \varepsilon(\omega) - j\sigma/\omega, \quad (1)$$

where $\varepsilon(\omega)$ is the frequency-dependent relative permittivity and σ is the conductivity of the medium [25]. $\varepsilon(\omega)$ is a complex number and can be described by many equivalent models, such as the Debye model or Lorentz model. In our case, the pulse duration is much longer than the relaxation time of water molecules (~ 10 ps), so $\varepsilon(\omega)$ can be considered as a constant, dispersionless value, ε_s (78) [26].

The conductivity was obtained from the resistance in a standard cuvette filled with cell medium by applying a 100 ns pulse and measuring the voltage and current. We can then calculate the conductivity from a known formula:

$$\sigma = d/AR, \quad (2)$$

where d is the gap distance and A is the electrode area. For the cuvette we used $d = 2$ mm and $A = 2$ cm². R was found to be 6.7 Ω and so σ is (1.5 S/m).

The complex permittivity can therefore be expressed as:

$$\varepsilon_r = \varepsilon_0 \varepsilon_s - jd/AR\omega \quad (3)$$

For a given input voltage, the electric field on the surface of the cover slip can be varied by raising the electrodes 50 μm , 100 μm and 150 μm above the cover slip. Since we set the input voltage of the coaxial cable as 1 V, the peak voltage across the parallel electrodes was calculated to be 0.88 V due to some reflection loss of the electrode configuration transition from the coaxial cable to the parallel electrodes. The electric field on the cover slip is shown in Fig. 5, and was measured at the center region to be 3.2 kV/m, 2.0 kV/m and 1.3 kV/m respectively. For all three electrode heights, the electric field in the center region is homogenous. The area covers approximately 50 $\mu\text{m} \times 70 \mu\text{m}$ for 50 μm , and 60 $\mu\text{m} \times 70 \mu\text{m}$ for both 100 μm and 150 μm . The cells in this region were used for study. For lower conductivities (0.5 S/m and 1 S/m), the electric field at the center region is 25% and 10% higher.

It was noticed that the FWHM (Full Width at Half Maximum) of the electric field pulse on the cover slip is broader than the pulse applied at the coaxial input (Fig. 6). This is due to the impedance discontinuity at the exit of the coaxial cable at the position close to the cover slip (Fig. 7a). Prior to 1 ns (Fig. 7b), the pulse already experiences an impedance discontinuity twice: one at the junction between the coaxial cable and the epoxy section that contains the rod electrodes and the other between the epoxy section and medium. At the end of the electrodes, one more reflection occurs at a time of about 1 ns. The reflected wave propagates along its original path but is reflected again at the junction between the water and epoxy section. Further, a reflection occurs once again at the junction between the coaxial cable and epoxy section. Despite these reflections, the total pulse width is still approximately 500 ps at the cell location. The pulse waveforms were found to be almost identical when the conductivity was varied from 0.5 S/m to 1.5 S/m. We note that the pulse broadening is mainly due to the water-submerged part of the tungsten rod electrodes, which have a length 3.5 mm. This contributes to a round trip time of approximately 200 ps for pulses traveling in water. Hence, the accurate measurement of rod electrode length is important in characterizing the actual pulse width. An error of 1mm length could result in a deviation of 58 ps of calculation from the actual pulse width seen at the cell location. On the other hand, the pulse broadening due to the epoxy section is negligible as the epoxy has a low dielectric constant (approximately 2.5). As such, the two conductors can be slightly off the axis of the coaxial cable and don't have to be strictly aligned with the axis of the coaxial cable. In general, the electrodes can be treated as two points, i.e., the edges of the tilted electrodes, so the electric field intensity on the cover slip is not sensitive to the tilt angle. Rather, it is determined by the distance between the two electrodes and the distance between the electrode bottom and the cover slip.

4. Biological Studies

a) Cell culture, plasma membrane voltage recordings, and data analysis

To demonstrate the efficacy of our exposure system, we monitored the change of transmembrane potential of murine neuroblastoma-rat glioma hybrid NG108 cells obtained from the American Type Culture Collection (ATCC, Manassas, VA). They were propagated at 37 °C with 5% CO₂ in air according to the supplier's recommendations and cultured in pyruvate free Dulbecco's Modified Eagle's medium (Caisson Labs, North Logan, UT),

supplemented with 4 mM L-glutamine, 4.5 g/l glucose, 10% FBS, 0.2 mM hypoxanthine, 400 nM aminopterin, and 0.016 mM thymidine. Cells were transferred onto “0” thickness glass cover slips 12–24 hours before experiments.

To characterize the changes of plasma membrane potential, Di-8-Anepps, a voltage-sensitive fluorescent dye, was used. To load Di-8-Anepps, cells on the cover slips were incubated in the physiological solution with 20 μ M of dye and 0.02% of Pluronic F-127 (Life Technologies, Grand Island, NY) for 45 min at 4 °C. External dye was then washed off and cover slips were transferred into a glass-bottomed perfusion chamber (Warner Instruments, Hamden, CT) mounted on an IX71 inverted microscope (Olympus America, Center Valley, PA). To be able to calibrate Di-8-Anepps and monitor real voltage changes we used ratiometric recordings. Dye was excited alternatively at 440 and 530 nm using a fast wavelength switcher Lambda DG4 (Sutter Instruments, Novato, CA). Emission was measured at 605 nm with an iXon Ultra 897 back-illuminated CCD Camera (Andor Technology, Belfast, UK). Voltage measurements typically began 2 seconds prior to subnanosecond exposure at a rate of 100 sampling points/s and continued for 20 s. Each cell or a group of cells were exposed only once to a single subnanosecond pulse. Individual traces were smoothed with a FFT filter utility of Origin 8.0 software.

In separate experiments, we also utilized patch clamp recordings to confirm plasma membrane voltage changes recorded with Di-8-Anepps. The plasma membrane potential was monitored in current-clamp zero current injection ($I=0$) mode using an Axopatch 200B amplifier, Digidata 1440A A–D converter, and pCLAMP10 software (MDS, Foster City, CA). Recording pipettes were manufactured by pulling borosilicate glass (BF150-86-10, Sutter Instrument) to a tip resistance of 1.5 MOhm. Recordings started 400 ms before subnanosecond pulse exposure and continued for 20 s after one pulse was applied.

b) Single Pulse effect on the cell membrane

Fig. 8 shows the plasma membrane potential changes after application of single subnanosecond pulses. In Fig. 8a, we used a voltage-sensitive dye to observe potential changes upon pulse application. After exposure to a 190 kV/cm electric field pulse, the cell's plasma membrane was depolarized by 15–20 mV. A similar effect was observed using the Patch-Clamp approach (Fig. 8b). As time elapsed, the membrane potential returned to its resting level (–60 to –70 mV) (not shown in the figure). The average membrane potential increased by 15–20 mV immediately after application of the pulse (Fig. 9).

5. Discussion

In the study of the interaction of biological specimen and electric field, homogenous electric fields are mostly generated by parallel plates, such as in cuvettes or Transverse Electromagnetic (TEM) cells. These systems are relatively large in physical size and the electric fields are accordingly low and usually limited by the breakdown strength of air. A system adaptable to a microscope, however, needs to be much smaller. It is well known that the electric field distribution between two parallel cylinders is relatively homogeneous. We show through modeling that, when cylindrical electrodes (rods) are used, the electric field projected on a cover slip from the centerpart region of the electrodes is still sufficiently

homogeneous for the study of cell response. Because the electrodes are submersed into a water-based medium, the hold-off voltage is limited by the breakdown strength of water, which is orders of magnitude higher than that of air [27]. In our experiments, we were therefore able to apply electric fields up to 190 kV/cm without observing any electrical breakdown between electrodes.

The circuit inductance, which defines the rise time of the pulses, was minimized by placing a series of resistors in a hollow tube, which has the same diameter as the insulation layer of the cable. The compactness of the assembly enables the π network to have a wide bandpass (up to 3 GHz) and a return loss (-17dB), which ensures a 500 ps single pulse exposure after absorbing most reflection caused at the junction of the coaxial cable and the electrodes. Surprisingly, even low power resistors ($1/4\text{ W}$ or $1/2\text{ W}$) in a simple serial and parallel combination could allow the network to work at a maximum voltage up to 16 kV.

The increase of transmembrane potential is generally caused by the opening of voltage-gated channels (such as VG-Na) or membrane poration. The depolarization of the cell's plasma membrane could then be due to the cation influx from the extracellular medium. As Na^+ is the most abundant ion in the extracellular solution (140 mM), it is highly likely that the depolarization is the result of Na^+ uptake. However, up to this point, it is still unclear which mechanism is responsible for membrane poration. The transmembrane voltage required for poration ranges from 200 mV to 1.4 V, while lower voltage may necessitate the VG channels opening. For pulses only lasting less than 1 ns, the charging process won't be adequate to induce a significant voltage change across the membrane due to the short duration. Rather, the electric field directly affects membrane lipids and membrane proteins as well as water dipoles.

The current flow through the cells (including plasma membrane and cytoplasm), medium and electrodes is conserved largely in terms of displacement current (not conduction current), provided that the pulse duration is shorter than the dielectric relaxation time of the cytoplasm ($\epsilon_0\epsilon_{\text{cp}}/\sigma_{\text{cp}}$), where ϵ_{cp} is the permittivity of the cytoplasm and σ_{cp} its conductivity [17]. Our pulse duration, 500 ps, is shorter than this time constant, which is approximately 700 ps for $\epsilon_{\text{cp}} = 78$ and $\sigma_{\text{cp}} = 1\text{ S/m}$, so the interaction of pulse and cell is shifted towards the dielectric phase. At the interfaces from the medium to the membrane as well as the cytoplasm, the electric displacement ($D = \epsilon_r\epsilon_0 E$) (considering that the conductive component of the medium and cytoplasm can be neglected) is continuous and the electric field in the membrane is amplified by a factor of 7, the ratio of the dielectric constants in cytoplasm/medium and membrane. The actual electric field in the cell membrane could be higher (20 times) by other estimates [28]. The increased field might then be sufficient for the membrane to be electroporated.

For the threshold electric field in our case, 190 kV/cm, the electric field strength in the membrane could reach 1.33 MV/cm. In molecular dynamics (MD) studies, a method which yields insights of how pores are created in a clean lipid bilayer, an electric field near 5 MV/cm, higher than that in our study, was found sufficient to create pores [29, 30]. However, MD modeling did not take membrane proteins into account, which might decrease the stability of the lipid bilayer and reduce the threshold electric field for pore formation. It

is likely that the proteins, such as those embedded in the VG channels, may experience a conformational change and therefore force the gates to open directly due to the ultra-high electric fields. Another potential effect that hasn't been considered in the MD calculations is the selective heating of the membrane by ultrashort pulses due to a dielectric relaxation process in the plasma membrane, which again might cause faster pore formation at lower membrane voltages [31].

6. Conclusion

A subnanosecond electric pulse exposure system for biological cells was constructed. It allowed us to study the bioelectric effect of pulses with electric field intensities as high as 190 kV/cm, a value that is not possible to reach using conventional TEM cells. The homogeneous region on the cover slip is approximately $50\text{ }\mu\text{m} \times 70\text{ }\mu\text{m}$, which is adequate to cover 5 biological cells of 10 μm in diameter. When applying a 300 ps long pulse to the network, pulse broadening was observed due to the discontinuity of impedance from the coaxial cable to the two parallel rod electrodes. This resulted in a 500 ps long pulse at the location of the cells on the cover slip of the microscope. The transmembrane potential of cells on the cover slip changed after the pulse exposure in neuroblastoma cells (NG108). Two methods, patch clamp and ratiometric fluorescence, were used to monitor the real-time potential change of the cells. Both methods provided the same level of potential change. For the first time, we have shown that a single subnanosecond (500 ps) electric pulse can increase cell membrane potential by approximately 20 mV at an electric field of 190 kV/cm. While we have shown single subnanosecond pulses to be effective in depolarizing membrane potential, an electric field of 190 kV/cm is still quite high and therefore poses a technical challenge for pulse delivery with wideband antennas. Future work will therefore focus on approaches to lowering the electric field.

Acknowledgments

This work was supported by an R21 grant (1R21EB016912-01A1) from the National Institute of Biomedical Imaging and Bioengineering, NIH. The authors would like to acknowledge with great appreciation the editing of the manuscript by Ms. Hollie Ryan.

References

1. Weaver, JC., Chizmadzhev, Y. Electroporation, Handbook of Biological Effects Electromagnetic Fields. Barnes, F., Greenebaum, B., editors. CRC Press; 2007.
2. Gowrishankar TR, Weaver JC. Electrical behavior and pore accumulation in a multicellular model for conventional and supra-electroporation. *Biochem Biophys Res Commun.* 2006; 349:643–653. [PubMed: 16959217]
3. Zimmermann U. Electric field-mediated fusion and related electrical phenomena. *Biochim Biophys Acta.* 1982; 694:227–277. [PubMed: 6758848]
4. Tsong TY. Electroporation of cell membranes. *Biophys J.* 1991; 60:297–306. [PubMed: 1912274]
5. Teissie J, Rols MP. Manipulation of cell cytoskeleton affects the lifetime of cell membrane electroporabilization. *Ann NY Acad Sci.* 1994; 720:98–110. [PubMed: 8010657]
6. Frey W, White JA, Price RO, Blackmore PF, Joshi RP, Nuccitelli R, Beebe SJ, Schoenbach KH, Kolb JF. Plasma membrane voltage changes during nanosecond pulsed electric field exposure. *Biophys J.* 2006; 90(10):3608–3615. [PubMed: 16513782]
7. Cole KS. Electric impedance of marine egg membranes. *Trans Faraday Soc.* 1937; 23:966.

8. Schoenbach KH, Beebe SJ, Buescher ES. Intracellular effect of ultrashort electrical pulses. *Bioelectromagnetics*. 2001; 22(6):440–448. [PubMed: 11536285]
9. Pakhomov AG, Kolb JF, White JA, Joshi RP, Xiao S, Schoenbach KH. Long-lasting plasma membrane permeabilization in mammalian cells by nanosecond pulsed electric field (nsPEF). *Bioelectromagnetics*. 2007; 28:655–663. [PubMed: 17654532]
10. Kotnik T, Miklavcic D. Second-order model of membrane electric field induced by alternating external electric fields. *IEEE Trans Biomedical Engineering*. 2000; 47(8):1074–1081.
11. Kotnik T, Miklavcic D. Theoretical evaluation of the distributed power dissipation in biological cells exposed to electric fields. *Bioelectromagnetics*. 2000; 21:385–394. [PubMed: 10899774]
12. Cobb BL, Jauchem JR, Mason PA, Dooley MP, Miller SA, Zirix JM, Murphy MR. Neural and behavioral teratological evaluation of rats exposed to ultra-wideband electromagnetic fields. *Bioelectromagnetics*. 2000; 21:524–537. [PubMed: 11015117]
13. Jauchem JR, Seaman RL, Lehnert H, Mathur SP, Ryan KL, Frei MR, Hurt WD. Ultra-wideband electromagnetic pulses: lack of effects on heart rate and blood pressure during two-minute exposures of rats. *Bioelectromagnetics*. 1998; 19:330–333. [PubMed: 9669547]
14. Jauchem JR, Frei MR, Ryan KL, Merritt JH, Murphy MR. Lack of effects on heart rate and blood pressure in ketamine-anesthetized rats briefly exposed to ultra-wideband electromagnetic pulses. *IEEE Trans Biomed Eng*. 1999; 46:117–120. [PubMed: 9919833]
15. Walters TJ, Mason PA, Sherry CJ, Steffen C, Merritt JH. No detectable bioeffects following acute exposure to high peak power ultra-wide band electromagnetic radiation in rats. *Aviat Space Environ Med*. 1995; 66:562–567. [PubMed: 7646407]
16. Rogers WR, Merritt JH, Comeaux JA, Kuhnel CT, Moreland DF, Teltschik DG, Lucas JH, Murphy MR. Strength-duration curve for an electrically excitable tissue extended down to near 1 nanosecond. *IEEE Transactions on Plasma Science*. 2004; 32:1587–1599.
17. Schoenbach KH, Xiao S, Joshi RP, Camp JT, Heeren T, Kolb JF, Beebe SJ. The effect of intense subnanosecond electrical pulses on biological cells. *IEEE Trans Plasma Science*. 2008; 36(2):414–422.
18. Camp JT, Jing Y, Zhuang J, Kolb JF, Beebe SJ, Song J, Joshi RP, Xiao S, Schoenbach KH. Cell death induced by subnanosecond pulsed electric fields at elevated temperatures. *IEEE Trans Plasma Science*. 2012; 40(10):2334–2347.
19. Hua YY, Wang XS, Zhang Y, Yao CG, Zhang XM, Xiong ZA. Intense picosecond pulsed electric fields induce apoptosis through a mitochondrial-mediated pathway in HeLa cells. *Molecular Medicine Reports*. 2012; 5:981–987. [PubMed: 22307872]
20. Xiao S, Guo S, Nesin V, Heller R, Schoenbach KH. Subnanosecond electric pulses cause membrane permeabilization and cell death, *IEEE Trans. Biomedical Engineering*. 2011; 58(5): 1239–1245.
21. Semenov I, Xiao S, Kang D, Schoenbach KH, Pakhomov AG. Cell stimulation and calcium mobilization by picosecond electric pulses. *Bioelectrochemistry*. 2015; 105:65–71. DOI: 10.1016/j.bioelechem.2015.05.013 [PubMed: 26011130]
22. Ji Z, Hagness SC, Booske JH, Mathur S, Meltz ML. FDTD analysis of a Gigahertz TEM cell for ultra-wideband pulse exposure studies of biological specimens. *IEEE TRANSACTIONS ON BIOMEDICAL ENGINEERING*. 2006; 53(5):780–789. [PubMed: 16686400]
23. Chretiennot, T., Catrain, A., Croizer, M., Vezinet, R. New concepts for the application of intense electromagnetic fields to biological samples. 1st World Congress on Electroporation and Pulsed Electric Fields in Biology, Medicine and Food & Environmental Technologies; Portoroz, Slovenia. September 6–10, 2015; 2015.
24. Barth, JE., Sarjeant, WJ. Direct subnanosecond voltage monitors. Digests of Technical Papers; IEEE Pulsed Power conference; 1981. p. 171
25. Allen, B., Dohler, M., Okon, EE., Malik, WQ., Brown, AK., Edwards, DJ. Ultra-wideband antennas and propagation for communications, radar and imaging. Vol. Chapter 20. Wiley; 2007.
26. Shahida Midi N, Sasaki K, Ohyama R, Shinyashiki N. Broadband complex dielectric constants of water and sodium chloride aqueous solutions with different DC conductivities. *IEEJ Transactions on Electrical and Electronic Engineering*. 2014; 9(S1):S8–S12.

27. Kolb JF, Joshi RP, Xiao S, Schoenbach KH. Streamers in water and other dielectric liquids. *J Phys D: Appl Phys.* 2008; 41:234007.
28. Vernier, PT. Nanoscale restructuring of lipid bilayers in nanosecond electric fields. In: Pakhomov, AG, Miklavcic, D., Markov, M., editors. *Advanced Electroporation Techniques in Medicine and Biology.* CRC Press; Boca Raton, FL: 2010. p. 161-176.
29. Vernier PT, Ziegler MJ, Sun Y, Chang WV, Gundersen MA, Tieleman DP. Nanopore formation and phosphatidylserine externalization in a phospholipid bilayer at high transmembrane potential. *J Am Chem Soc.* 2006; 128(19):6288–6289. [PubMed: 16683772]
30. Delemotte L, Tarek M. Molecular dynamics simulations of lipid membrane electroporation. *J Membrane Biol.* 2012; 245:531–543. [PubMed: 22644388]
31. Croce RP, De Vita A, Pierro V, Pinto IM. A thermal model for pulsed EM field exposure effects in cells at nonthermal levels. *IEEE Transactions on Plasma Science.* 2010; 38(2):149–155.

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Fig. 1.

A π resistor network serves as an attenuator and absorbs the reflections from the two electrodes submerged in cell culture medium. a) The photo of the resistor network; b) The detailed resistor arrangement. Because the resistors were configured in a symmetrical layout, the resistance viewed from the left port is identical to that viewed from the right port.

Fig. 2.

The magnitude of S_{11} of the π resistor network. Up to 3GHz, the reflection coefficient stays relatively constant, approximately -17 dB, close to its ideal value -18 dB. Beyond 3 GHz, the reflection loss becomes larger and deviates from its ideal value.

Fig. 3.

Test setup for the π -network. A subnanosecond pulse generator was connected to the 20 X Barth attenuator through the π network. a) Pulse waveforms read from three probes: 1) the voltage pickup by the FID probe; 2) the voltage from the Barth probe; and 3) a Farr V-dot probe. The oscilloscope was a 20 GHz bandwidth oscilloscope (Tektronix TDS7404). b) The pulses measured from the three probes.

Fig. 4.

The electrodes, consisting of two tungsten rods (diameter: 100 μm , gap distance: 170 μm), were connected to a coaxial cable (RG316), which was fixed on a brass rod and further mounted on a micromanipulator. The other side of the coaxial cable was connected to the π network. The lines 1–3 indicate the locations where reflections occur before the pulse reaches the end of the electrodes.

Fig. 5.

The electric field distribution for the electrode height of 50 μm , 100 μm and 150 μm . The diameter of the electrodes is 100 μm and the gap distance is 170 μm . The input voltage was 1 V.

Fig. 6.

A Gaussian pulse, corresponding to the measured pulse shape (Fig. 3b), was used as the input of the coaxial cable in the simulation, which allowed us to calculate the field to which the cells on the cover slip were exposed. For an input voltage of 1 V, the peak electric fields on the cover slip at the center point beneath the electrodes were 3.2 kV/m, 2.0 kV/m and 1.3 kV/m for the electrode tips positioned at 50 μm , 100 μm and 150 μm above the coverslip.

Fig. 7.

(a) Side view of the coaxial cable in the simulation. (b) Multiple impedance discontinuities cause reflections and broadening of the pulse: the breakout of the coaxial cable (indicated by dash line 1); the junction between the coaxial cable dielectrics and epoxy section (dash line 2); and the junction between water and epoxy section (dash line 3). The electrodes, cover slip and epoxy section are fully submerged in water.

Fig. 8.

A typical measurement of transmembrane potential in a single NG108 cell after exposure to a single subnanosecond pulse. (a) The result measured by ratiometric fluorescence imaging using Di-8-Anepps. (b) The result measured by patch-clamp.

Fig. 9.

The averaged change of transmembrane potential of NG108 cells measured by ratiometric fluorescence imaging (n=18) and patch-clamp (n=9).