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Modified Blumlein Pulse-Forming Networks for Bioelectrical Applications

Stefania Romeo · Maurizio Sarti · Maria Rosaria Scarfì · Luigi Zeni

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Abstract Intense nanosecond pulsed electric fields (nsPEFs) have been shown to induce, on intracellular structures, interesting effects dependent on electrical exposure conditions (pulse length and amplitude, repetition frequency and number of pulses), which are known in the literature as "bioelectrical effects" (Schoenbach et al., IEEE Trans Plasma Sci 30:293–300, 2002). In particular, pulses with a shorter width than the plasma membrane charging time constant (about 100 ns for mammalian cells) can penetrate the cell and trigger effects such as permeabilization of intracellular membranes, release of Ca^{2+} and apoptosis induction. Moreover, the observed effects have led to exploration of medical applications, like the treatment of melanoma tumors (Nuccitelli et al., Biochem Biophys Res Commun 343:351–360, 2006). Pulsed electric fields allowing such effects usually range from several tens to a few hundred nanoseconds in duration and from a few to several tens of megavolts per meter in amplitude (Schoenbach et al., IEEE Trans Diel Elec Insul 14:1088–1109, 2007); however, the biological effects of subnanosecond pulses have been also investigated (Schoenbach et al., IEEE Trans Plasma Sci 36:414–422, 2008). The use of such a large variety of pulse parameters suggests that highly flexible pulse-generating systems, able to deliver wide ranges of pulse durations and amplitudes, are strongly required in order to explore effects and applications related to different exposure conditions. The Blumlein pulse-forming network is an often-employed circuit topology for the generation of high-voltage electric pulses with fixed pulse duration. An innovative modification to the Blumlein circuit has been recently devised which allows generation of pulses with variable amplitude, duration and polarity. Two different modified Blumlein pulse-generating systems are presented in this article, the first based on a coaxial cable configuration, matching microscopic slides as a pulse-delivery system, and the other based on microstrip transmission lines and designed to match cuvettes for the exposure of cell suspensions.

S. Romeo (✉) · L. Zeni
Department of Information Engineering, Second University of Naples, via Roma 29, Aversa, Italy
e-mail: stefania.romeo@unina2.it

M. Sarti · M. R. Scarfì
CNR–Institute for Electromagnetic Sensing of the Environment (IREA), via Diocleziano 328, 80124 Naples, Italy

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Introduction

The application of nanosecond pulsed electric fields (nsPEFs) to cells and tissues has gained considerable attention over the last decade, due to the very interesting effects observed at various levels of cell morphology and function, which have disclosed new and fascinating scenarios in both biological and medical fields. In parallel to experimental advances, cell electrical models have also been developed in order to explain the interaction mechanisms underlying the observed phenomena.

Schoenbach et al. (2001) have shown that a number of pulses with 60 ns duration and 3.6 and 5.3 MV/m amplitudes, applied to human eosinophils, labeled with calcein as an intracellular probe, are able to induce selective poration/disruption of intracellular membranes without loss of plasma membrane integrity, while Buescher et al. (2004)

indicated dose–response effects in the variations of intracellular [Ca^{2+}] induced by 300 and 60 ns pulses in Fluo3-loaded human neutrophils. Further experiments have indicated the possibility of triggering important cell functions by means of different exposure conditions to nsPEFs and on different cell types. In particular, changes in intracellular [Ca^{2+}] were investigated by Kolb et al. (2008) on individual Jurkat cells as a response to nsPEFs of 60 ns pulse duration with field strengths of 2.5, 5 and 10 MV/m and by Vernier et al. (2008) in excitable bovine adrenal chromaffin cells exposed to 4 ns electric pulses with field intensities ranging 2–8 MV/m. This is a remarkable effect since the release of calcium from internal cell stores can activate cellular processes such as apoptosis, which has implications for cancer therapy.

Moreover, Beebe et al. (2003) have shown the kinetics of nsPEF-induced apoptosis in human cells to be dependent on the pulse duration: As the pulse duration increases, within the 10–300 ns range, multiple and synergistic mechanisms are involved, which make apoptosis progression faster.

Although the mechanisms of action determining the selective interaction between ultrashort pulses and intracellular structures need further and thorough study, experimental evidence obtained to date suggests that the response of particular cell types to nsPEFs depends on the electrical exposure parameters (pulse rise time, amplitude and duration, number and frequency of applied pulses). In particular, the immediate biophysical response is determined by the duration and strength of the applied electric field, while the secondary biological response is additionally controlled by the number of pulses applied and their repetition rate during the exposure (Kolb et al. 2008). Accordingly, the availability of highly flexible and strictly controllable pulse-generating systems is a crucial point in order to explore the numerous possible exposure conditions and the related biological effects. In this respect, pulse generator configurations based on transmission lines (Blumlein pulse-forming network, PFN) are often employed in which pulse duration and polarity are fixed by the physical parameters of the line (length, impedance, etc.) and cannot be modified without changing the line itself. An innovative modification to the classical Blumlein topology (modified Blumlein, Fig. 1) has been recently introduced by de Angelis and coworkers (de Angelis et al. 2006). As in the classical configuration, the modified Blumlein is able to deliver, to a matched load, a voltage pulse whose amplitude is set by the voltage power supply used, while, conversely to the previous one, the pulse duration and polarity are changeable by means of the synchronization of switches. The longest possible pulse duration is given by the electrical length of the line, while the shortest one is stated by the control component performance (de Angelis et al. 2006). Such a configuration has been designed and tested in order to generate pulses with amplitude up to 1 kV and variable durations between 8 and 60 ns; the modified Blumlein gave good results in terms of both flexibility and the reduction of pulse distortion due to nonlinear characteristics affecting pulse shape in the classic Blumlein configuration, which were compensated for by the symmetry of the modified system (de Angelis et al. 2008). Further modifications to the modified Blumlein PFN have been reported by Rebersek et al. (2009), who developed a four-switches Blumlein configuration, which enables a higher repetition rate of variable duration of either unipolar or bipolar high-voltage pulses.

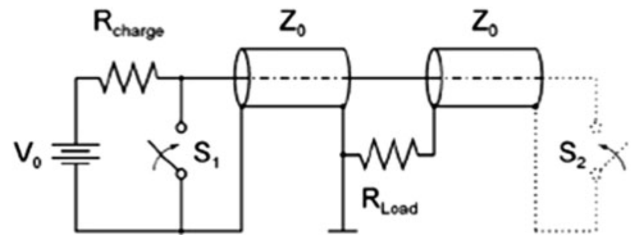

Fig. 1 Modified Blumlein PFN. The double switch configuration allows one to vary the pulse duration and polarity without changing the physical characteristics of the line. Pulse duration is stated by the delay between the signals triggering the switches closure, while it is possible to change the polarity by reversing the trigger sequence. Moreover, the symmetrical circuit topology allows one to overcome distortions arising from the use of nonideal components, which in the classical configuration give rise to a residual voltage across the load after the desired pulse duration

In the present work, we describe the design and realization of a modified Blumlein PFN based on coaxial cable transmission lines that is able to deliver pulses ranging 30–200 ns in duration and up to 1 kV in amplitude to cell suspensions placed on microscope slides (with a gap of 100 μm between the electrodes so that an electric field strength of up to 10 MV/m can be achieved). Moreover, the design of a modified Blumlein based on a microstrip line configuration is also reported. In the latter case, numerical results will be presented concerning a more compact structure matching electroporation cuvettes as exposure system, modeled and designed for the generation of pulses with variable durations up to 50 ns and amplitude higher than 1 kV.

The setup of two pulse-generating systems, each covering a wide range of pulse durations and amplitudes and matching two different and complementary pulse-delivery systems, is aimed at biological experiments exploring several exposure conditions to the nsPEFs and to investigate the related bioelectrical effects. The final goal is to tailor nsPEFs to achieve selectivity, with respect not only to the desired cellular response but also to the cell type being targeted (Vernier et al. 2008).

Materials and Methods

Coaxial Cable-Modified Blumlein PFN

The circuit was simulated and designed using OrCad PSpice 9.1 software (Cadence Design Systems, San Jose, CA).

The pulse generator is expected to deliver square pulses of up to 1 kV peak amplitude and ranging in length from 30 to 200 ns, to a narrow channel of cells on a microscope slide. The Blumlein line consists of two identical series connected RG58 (50 Ω) coaxial cables (each 20 m long, with an input-to-output delay time of about 5 ns/m) charged to a common voltage. Two fast RF power MOSFET switches (DE275-102N06A; Ixys, Fort Collins, CO) were employed for the pulse length variation, characterized by a closing time of about 2 ns, a nominal residual resistance in the on state of 1.6 Ω and the ability to hold a maximum voltage of 1 kV. The switches were placed on a couple of evaluation boards (EVIC 420; DEI, Ixys Corporation, Fort Collins, CO), where the gate driver chips (DEIC 420, DEI, Ixys Corporation, Fort Collins, CO) were also mounted. Two 200 kΩ equivalent resistances were inserted in both sides of the circuit in order to limit the nominal current in the switches to 5 mA. All these components were mounted in a metallic box whose frame acts as the ground plane for the whole circuit (Fig. 2). The MOSFETs synchronization was performed by means of a couple of digital computer-controlled pulse generators (USB pulse 100; Elan Digital System, Fareham, UK), which are able to apply pulse trains of 1.5–5 V in amplitude and variable length (from 10 ns to 10 s) and frequency (up to 100 MHz) to the MOSFET gate drivers through a TTL input.

In order to match the Blumlein line to the load, microreactors were used, imposing an equivalent impedance of 100 Ω. It is well known that, in the Blumlein line, in order to deliver a pulse amplitude to the load equal to the applied voltage, each individual line must exhibit a characteristic impedance equal to one-half the impedance of the load. Accordingly, the microreactors were designed following the simple formula

$$R_L = \rho \frac{d}{A} = 100\,\Omega \tag{1}$$

where the resistivity, ρ, of the medium in which cells are suspended is 100 Ωcm (Kolb et al. 2006). Assuming for the electrodes a length of 1 cm and a thickness of 100 μm, so that the area, A, is 0.01 cm^2, a gap distance, d, of 100 μm is necessary in order to have the desired impedance. Following these statements, stainless-steel electrodes, spaced 100 μm apart, were glued onto microscope slides so that a maximum electric field strength of 10 MV/m could be applied to the cells.

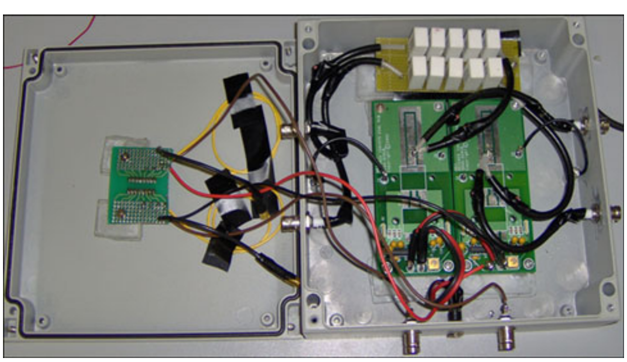

Fig. 2 Modified Blumlein PFN control circuit. MOSFET switches, gate drivers (both mounted on the downward faces of the evaluation boards) and limiting drain current resistances are placed in the metallic box

Microstrip Line-Modified Blumlein PFN

A modified Blumlein PFN was designed in a spiral microstrip line configuration in order to have a more flexible and compact stucture tought to match electroporation cuvettes as a pulse delivery system. Numerical results are presented in this work.

A strip spiral Blumlein using water as dielectric has been proposed by Liu et al. (2007) as a pulse-forming line for a high power–pulsed modulator. Such a configuration allowed longer pulses without reducing the compactness of the structure.

Concerning the biomedical applications of nsPEFs, planar configurations of the Blumlein PFN have also been reported in the literature. A linear stripline configuration for a classical Blumlein PFN was proposed by Kolb et al. (2006) in order to generate high-voltage nanosecond electric pulses. The stripline consisted of a couple of metallic foils separated by a dielectric layer, whose dimensions were stated by the following equations:

$$Z = \frac{\varsigma_0}{(\varepsilon_r)^{1/2}} \frac{t}{w} \tag{2}$$

where Z is the characteristic impedance of the line, ζ_0 is the vacuum characteristic impedance (about 377 Ω), ε_r is the dielectric constant of the insulating layer between the conducting strips, t is the thickness of the insulating layer and w is the width of the strips, and

$$\tau = 2l \frac{(\varepsilon_r)^{1/2}}{c} \tag{3}$$

τ being the pulse duration and l the stripline length (Kolb et al. 2006).

Following Eqs. 2 and 3, the structure was modeled and simulated by means of the Finite Integration method, with CST Microwave Studio® 5 (CST, Darmastadt, Germany).

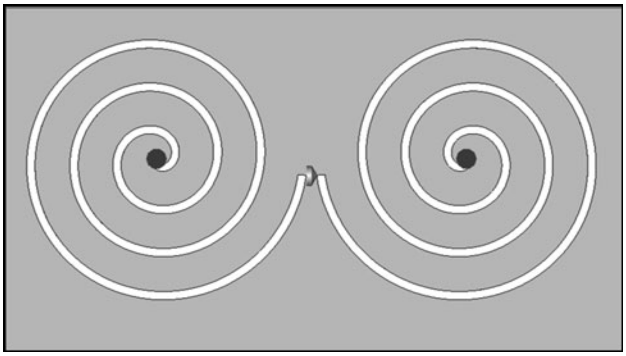

Fig. 3 Top view of the modified Blumlein in spiral microstrip line configuration. Strip width and thickness are 1.2 cm and 0.5 mm, respectively, while the distance between coils is about 5 cm. A resistive 100 Ω load is series-connected between the spirals, while two discrete ports are placed in the center of each *spiral*, between the *line* and the ground plane, in order to simulate the switches

As shown in Fig. 3, a metallic sheet acts as a ground plane, separated by the insulating layer from the two metallic (copper) transmission lines modeled in a spiral configuration. The load, simulated as a lumped element, is placed at the structure center, series connected to the metallic spirals, while the switches, simulated as voltage discrete ports, connect each spiral conductor to the ground plane through a hole in the dielectric layer. In order to simulate the switches' synchronization signals, two square pulses with the rise edge in common were applied, setting the desired delay, which states the pulse duration.

Since the transmission line length, l, is strongly dependent, besides the pulse duration, also on the dielectric constant of the insulating material, a proper choice of the latter is a critical point of the design. The higher the maximum desired pulse duration, the higher is l; but larger values for ε_r allow shortening of the spiral length so that a trade-off is required between the possibility of covering a wide range of pulse durations and the structure's compactness. Moreover, the choice of insulating material, through the dielectric strength parameter, determines the maximum applicable voltage value. The impedance matching the load sets the width and thickness of the layer.

As an example, a spiral microstrip line–modified Blumlein was simulated, which was matched to a 10 Ω load and was able to generate variable pulse durations up to 50 ns. Choosing aluminum oxide (Al_2O_3) as dielectric material ($\varepsilon_r = 9.8$) and thickness $t = 0.5$ mm for the insulating layer, the following line parameters were calculated:

$w = 1.2$ cm

$l = 239.6$ cm

In the spiral configuration, a more compact structure was obtained, taking up, on the whole, a volume of $90 \times 50 \times 0.1$ cm^3. The dielectric strength of Al_2O_3 being as large as 30 kV/mm, the structure can undergo a maximum voltage of 15 kV.

Results

The coaxial cable–modified Blumlein PFN has been tested in several conditions, showing satisfactory agreement with the simulations and good behavior in the generation of variable duration and polarity pulses, with a pulse rise time shorter than 20 ns. In Fig. 4a, b two cases of variable length pulses are reported (respectively, 100 and 150 ns full width at half-maximum, FWHM) with the relative activation signals, while Fig. 4c shows the positive 100 ns pulse, obtained by reversing the activation order of control signals. The performance at the boundary duration range conditions is shown in Fig. 5. The voltage across the load appears after a delay time, from the first switch closure, of about 200 ns, corresponding to the electric line length. A voltage attenuation of about 10% is registered across the load due to the voltage drop across the limiting current resistances. With regard to the spiral microstrip line configuration, numerical results are reported about the circuit behavior in ideal conditions using Al_2O_3 as the dielectric layer between the strips. In Fig. 6 positive and negative voltage pulses across the load are reported, together with the simulated switches' synchronization signals: The negative pulse was obtained by reversing the order of the synchronization signals.

Discussion

The design, realization and characterization of a coaxial cable–modified Blumlein circuit are presented. Good behavior, in terms of variability of pulse duration and polarity, was achieved in the pulse duration range of 30–200 ns, with the pulse rise time mainly affected by the propagation delays due to the control circuit components. The transmission line has a 50 Ω characteristic impedance in order to match 100 Ω microscope slides as a pulse-delivery system, obtained by mounting stainless-steel electrodes on glass microscope slides with a 100-μm gap in between, in which the cell suspension is placed during exposure to electric field strengths of up to 10 MV/m.

A spiral microstrip line configuration for a modified Blumlein network has also been proposed, obtaining a flexible behavior, as corroborated by numerical simulations, in a more compact structure than the coaxial cable one. In order to match this structure to a cuvette-based exposure system and to apply electric fields of several

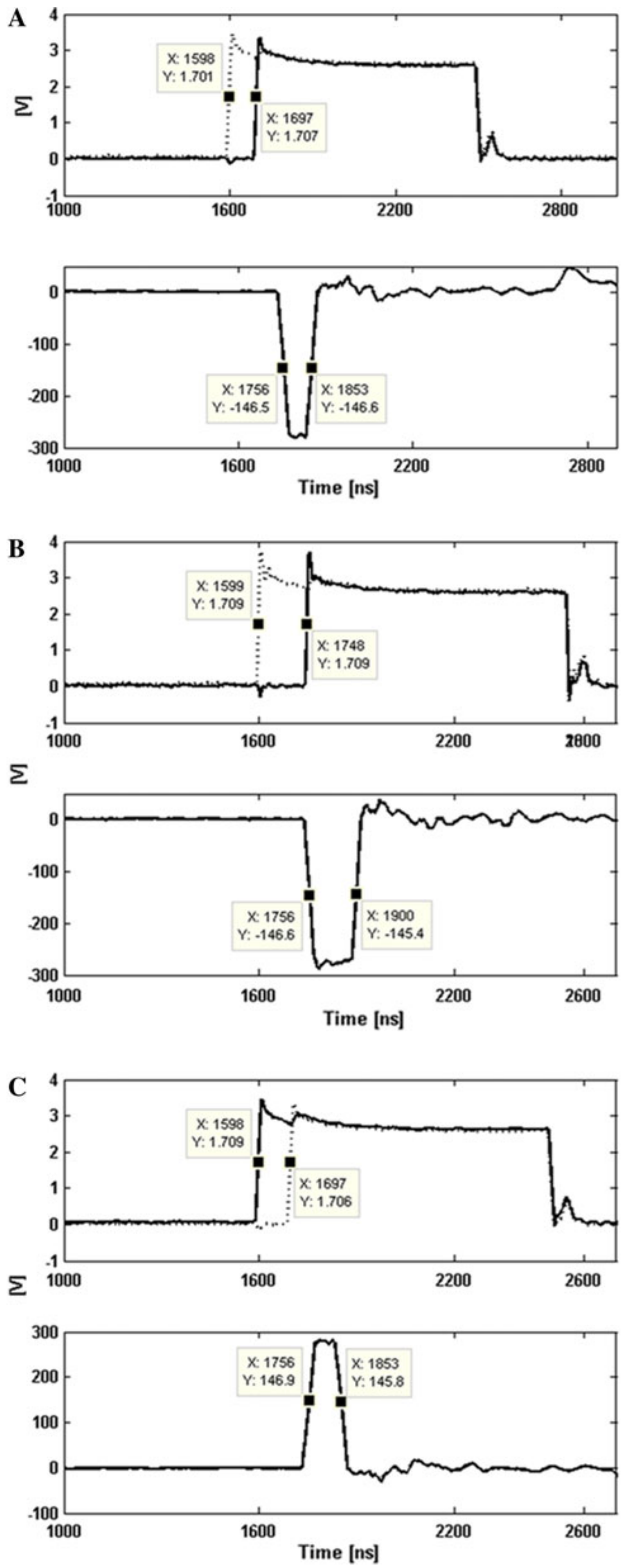

Fig. 4 Activation signals and voltage across the load. **a** Pulse duration is 100 ns (full width at half maximum, FWHM) due to the activation delay between the switches. **b** Pulse duration is 150 ns (FWHM) due to the activation delay between the switches. **c** Positive pulse due to the reversed switches activation order

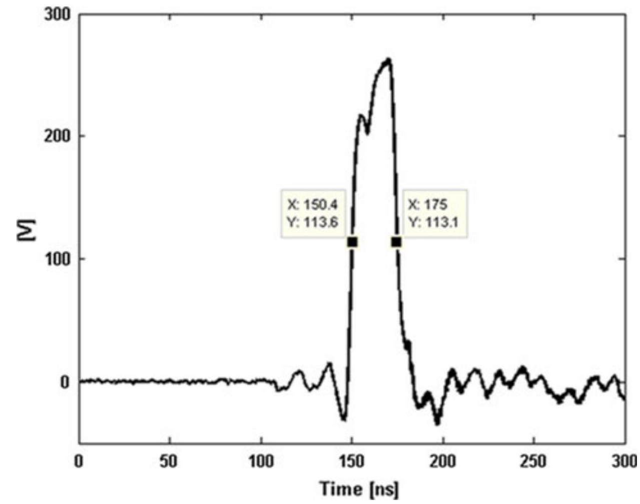

Fig. 5 Pulse duration of 30 ns: It is the shortest pulse duration possible with the proposed coaxial cable–modified Blumlein PFN

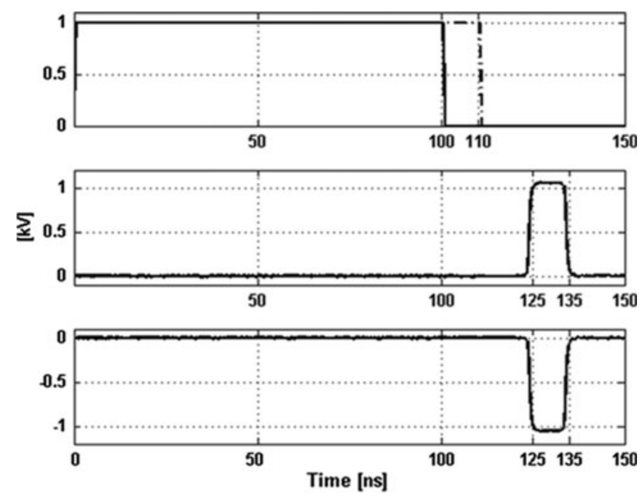

Fig. 6 From *top downward*, (*1*) simulated switch synchronization trigger sequence associated with the discrete ports in order to simulate the signals determining the line discharge and the pulse length, (*2*) 1 kV–10 ns pulse applied to the load in the numerical simulation of the spiral stripline Blumlein PFN due to the switches synchronization signals and (*3*) negative pulse obtained by reversing the switches activation order

ten of MV/m, the dielectric material needs to be properly chosen and high-voltage switches, such as spark gaps, must be employed.

The opportunity to treat biological samples with different nsPEF exposure conditions, changing the electrical parameter in the framework of the biological experiment itself, is a crucial point for understanding the mechanisms underlying the nsPEF effects on intracellular structures, which can be allowed by means of flexible pulse-generating systems.

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