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Microdosimetric Study for Nanosecond Pulsed Electric Fields on a Cell Circuit Model with Nucleus

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Abstract Recently, scientific interest in electric pulses, always more intense and shorter and able to induce biological effects on both plasma and nuclear membranes, has greatly increased. Hence, microdosimetric models that include internal organelles like the nucleus have assumed increasing importance. In this work, a circuit model of the cell including the nucleus is proposed, which accounts for the dielectric dispersion of all cell compartments. The setup of the dielectric model of the nucleus is of fundamental importance in determining the transmembrane potential (TMP) induced on the nuclear membrane; here, this is demonstrated by comparing results for three different sets of nuclear dielectric properties present in the literature. The results have been compared, even including or disregarding the dielectric dispersion of the nucleus. The main differences have been found when using pulses shorter than 10 ns. This is due to the fact that the high spectral components of the shortest pulses are differently taken into account by the nuclear membrane transfer functions computed with and without nuclear dielectric dispersion. The shortest pulses are also the most effective in porating the intracellular structures, as confirmed by the time courses of the TMP calculated across the plasma and nuclear membranes. We show how dispersive nucleus models are unavoidable when dealing with pulses shorter than 10 ns because of the large spectral contents arriving above 100 MHz, i.e., over the typical relaxation frequencies of the dipolar mechanism of the molecules constituting the nuclear membrane and the subcellular cell compartments.

Keywords Microdosimetry · Nanosecond pulsed electric field · Cell circuit model with nucleus

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Introduction

In recent times, scientific interest in extremely short (few nanoseconds) and intense (up to tens of megavolts per meter) electric pulses has greatly increased (Joshi and Schoenbach 2010). Biological experiments have demonstrated that such pulses, indicated as nanosecond pulsed electric fields (nsPEFs), could induce effects on both plasma and nuclear membranes, leading to the electric manipulation of the cell external membrane and internal organelles (Buescher and Schoenbach 2003; Chen et al. 2007; Vernier et al. 2008). The basic mechanisms of this process are not completely understood, and models at the molecular (molecular dynamic simulations) (Vernier et al. 2006; Marracino et al. 2013) and cellular (microdosimetry) levels are useful for a better comprehension of the phenomenon. In particular, microdosimetric models, including intracellular structures like the nucleus, have assumed increasing importance due to the useful information they are able to provide when planning experimental activities (Smith et al. 2006; Gowrishankar et al. 2006). Indeed, such models enable evaluation of the onset of the electroporation phenomenon on the basis of a fundamental quantity, the transmembrane potential (TMP). The value of the TMP permits us to establish the membrane poration; in particular, it occurs when this value exceeds the electroporation threshold of 1 V (Joshi and Schoenbach 2010). In the current literature, different types of microdosimetric

models are present: analytical, numerical and circuit models. The analytical models rely on the solution of the quasi-static Laplace equation on a spherical cell (Kotnik and Miklavcic 2006; Kotnik et al. 2010; Merla et al. 2011); the numerical ones are based on a distributed circuit representation (Joshi et al. 2004; Schoenbach et al. 2007), on a lattice transport network description (Stewart et al. 2004; Gowrishankar et al. 2006) or on a finite element method (Pucihar et al. 2009; Elia et al. 2010). Circuit models can be extremely advantageous (Schoenbach et al. 2007; Yao et al. 2008; Merla et al. 2012). They allow us to obtain rapid information about the TMP intensity in the membranes and, hence, information on the poration process. Furthermore, they enable a direct time domain analysis without the need of using Fourier transform, as in Merla et al. (2011), with a great simplification of the solution.

In microdosimetric models, the dielectric dispersion of cell compartments has often been disregarded; but a few recent articles have demonstrated the importance of considering detailed dispersive models when evaluating the TMP on the plasma membrane for pulses shorter than some nanoseconds (Joshi and Hu 2011; Merla et al. 2011).

In this article, a compact circuit model of the cell, including the nucleus, is proposed. All the cell compartments are modeled accounting for their dispersive behavior. The responses of the dispersive and nondispersive nucleus representations are compared.

Our final aim was to evaluate the influence of the nucleus modeling on the penetration inside the cell by the different types of nsPEF defined in Merla et al. (2012) in terms of the dielectric properties of its compartments and in terms of the intensity of the TMP induced on the external and internal cell membranes.

Materials and Methods

Cell Circuit Models with Nucleus

In this article, the topology of a cell circuit model with both dispersive and nondispersive nuclei is considered (Fig. 1). The proposed circuit is an extension of the one used in Merla et al. (2012) to treat a simple three-layered spherical cell. The circuit comprises a series of one-port elements for each cell compartment. In order to take into account the contribution of the current that flows around the membranes, the introduction of further parallel branches for the extracellular medium and the cytoplasm is necessary (Fig. 1; Yao et al. 2008).

The one-port element, when the dielectric dispersion is disregarded, is a simple shunt between a resistor (R_{si}) and a capacitor (C_{si}) (Polk and Postov 1995), describing only the contribution due to the static characteristics of the medium (ε_{si}, σ_i). These elements are dependent on the electrical properties of the compartments and their geometry and are calculated, per unit area, according to the following equations:

$$R_{si} = \frac{d_i}{\sigma_i}\left[\Omega\mathrm{m}^2\right] \tag{1}$$

$$C_{si} = \frac{\varepsilon_0 \varepsilon_{si}}{d_i}\left[\mathrm{F}/\mathrm{m}^2\right] \tag{2}$$

with i used to indicate the different cell compartments; d_i is the physical length where each circuit element is defined, depending on each cell compartment; ε_0 is the permittivity of the vacuum; ε_{si} and σ_i are the static permittivity and the conductivity of each compartment, respectively. In Table 1 actual values are reported. Particularly, d_i corresponds for the plasma and nuclear membrane to their thickness, for the cytoplasm to its dimension considering the presence of the nucleus, for the nucleoplasm to its diameter and for the extracellular medium to the formulation proposed in Wachner et al. (2002). The equivalent circuit for the nondispersive model of the nucleus is reported in the lowest inset of Fig. 1.

When the dielectric dispersion is considered, the simple shunt of R_i and C_i is integrated with a series of a resistor R_i^D and a capacitor C_i^D in order to include the dielectric relaxation phenomena of each cell compartment. The superscript D is used to indicate the circuit elements belonging to the so-called dispersive branch. The dispersive model of the cell is based on the introduction of a single-term Debye equation within the different compartments, and the single terms were calculated from the parameters of the Debye model for permittivity and conductivity (ε_{si}, $\varepsilon_{\infty i}$, τ_i, σ_i) with the following relationships (Polk and Postov 1995):

$$R_i = \frac{d_i}{\sigma_i}\left[\Omega\mathrm{m}^2\right] \tag{3}$$

$$C_i = \frac{\varepsilon_0 \varepsilon_{\infty i}}{d_i}\left[\mathrm{F}/\mathrm{m}^2\right] \tag{4}$$

$$C_i^D = \frac{\varepsilon_0(\varepsilon_{si} - \varepsilon_{\infty i})}{d_i}\left[\mathrm{F}/\mathrm{m}^2\right] \tag{5}$$

$$R_i^D = \frac{d_i \tau_i}{\varepsilon_0(\varepsilon_{si} - \varepsilon_{\infty i})}\left[\Omega\mathrm{m}^2\right] \tag{6}$$

where d_i is the physical length over which each circuit element is defined (Table 1). Finally, $\varepsilon_{\infty i}$ and $f_{ri} = 1/2\pi\tau_i$ are the well-known Debye parameters indicating the residual permittivity at high frequency and the relaxation frequency of each compartment, respectively (Polk and Postov 1995); $\varepsilon_{si} - \varepsilon_{\infty i}$ corresponds to the dispersion strength. These circuit components represent the physical mechanisms observed in the cell. In particular, C_i takes into account the faster polarization and orientation phenomena; R_i is a resistor, which considers the conductivity of the

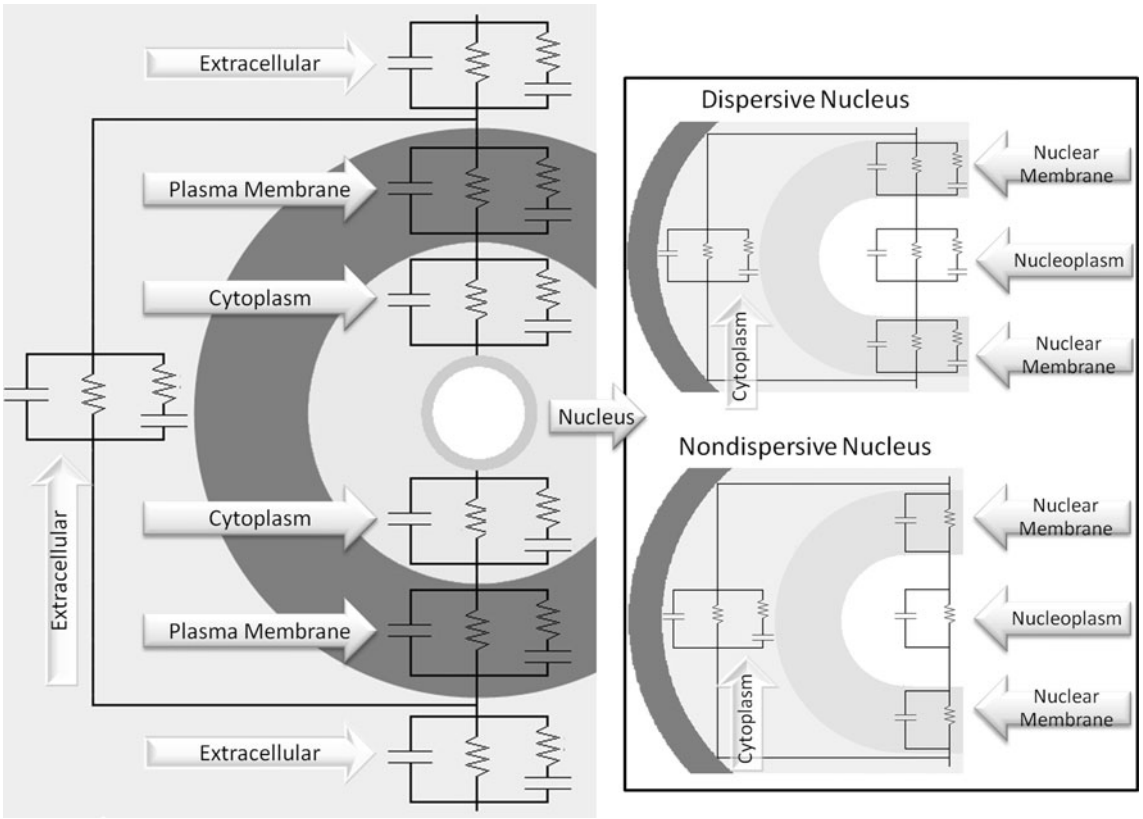

Fig. 1 Cell circuit model with dispersive and nondispersive nuclei

Table 1 Cell compartment dielectric properties

Compartment	ε_s	ε_∞	σ (S/m)	f_r (MHz)	d (μm)
Extracellular	67.00	5.00	0.55	17900	2.5
Plasma membrane	11.70	4.00	1.10×10^{-7}	179.85	0.01
Cytoplasm	67.00	5.00	0.55	17900	3.75
Nuclear membrane	11.70	4.00	8.30×10^{-5}	179.85	0.01
Nucleoplasm	67.00	5.00	0.55	17900	2.5

material; C_i^D is the polarization drop due to relaxation; and R_i^D incorporates the relaxation phenomenon.

The dielectric parameters of the cell compartments, except for the nucleus, are those assessed in Merla et al. (2009) with the technique well presented in Piuzzi et al. (2013) and reported in Table 1 using a single dispersion term for the different cell compartments. In that model the Debye membrane parameters were obtained by combining dielectric measurements of liposome solutions with a fitting algorithm on proper mixture theory formulas. For the extracellular medium and cytoplasm the same parameters were used; they were derived from measurements of phosphate-buffered saline solution at 27 °C.

Regarding the nucleus model, the membrane thickness is set equal to the plasma membrane, as suggested in Kotnik and Miklavcic (2006), with a value of 10 nm. The problem of the electrical parameters of the nuclear membranes and nucleoplasm is a topic not yet well settled in the literature; some extensive work has been done in order to identify minimum and maximum possible values (Ermolina et al. 2000). All the values chosen here are well inside those ranges. In particular, a conductivity of 8.30×10^{-5} S/m was chosen as the minimal value, as suggested in Ermolina et al. (2000). The static membrane permittivity is set at 11.7, in accordance with Merla et al. (2009), where the value was estimated for purely phospholipidic membranes, and is inside the range suggested in Ermolina et al. (2000).

The dispersive dielectric properties of the nuclear membrane and nucleoplasm have still not been studied and characterized; hence, as first step, they were chosen to be equal to the cell membrane ones. This choice is plausible since the relaxation phenomena of the nuclear membrane are likely due to the same polarization mechanisms of the plasma membrane, i.e., the dipole orientation and Maxwell and Wagner polarization, as well known and fully reported in Polk and Postov (1995). For the nucleoplasm, the same dispersive characteristics of a conductive solution were

Table 2 Cell nucleus dielectric properties as in Hu et al. (2005), Yao et al. (2008)

	Nuclear membrane		Nucleoplasm	
	ε_s	σ (S/m)	ε_s	σ (S/m)
Yao et al. (2008)	10	1.00×10^{-3}	80	1
Hu et al. (2005)	22.8	4.30×10^{-3}	120	0.18

Table 3 Pulse parameters in accordance with the current literature

Pulse	T_{on} (ns)	Rise time (ns)	Fall time (ns)	Amplitude (MV/m)
ES_nsPEF	1	0.1	0.1	9.20
VS_nsPEF	3	1.5	1.5	5.00
S_nsPEF	10	2	2	2.13
M1_nsPEF	60	2	2	0.50
M2_nsPEF	60	10	10	0.46
L_nsPEF	300	30	30	0.19

used, exploiting the hypothesis of Kotnik and Miklavcic (2006); all values are in Table 1.

Moreover, the results for nuclear TMP obtained with this model were compared with those obtained using two other electrical models for the nucleus (nondispersive) taken from the literature (Hu et al. 2005; Yao et al. 2008); the values are reported in Table 2. Our model presents values for the permittivity of the nuclear membrane in between the two models (Yao et al. 2008; Hu et al. 2005), while the membrane conductivity is lower.

Six different types of nsPEFs, classified as in Merla et al. (2012) in accordance with the current literature, were adopted as input signals of our simulations; the features of each signal category are tabulated in Table 3. For each pulse, we used the minimal amplitude of the extracellular field necessary to induce poration in the plasma membrane with a single trapezoidal pulse, as calculated in Merla et al. (2012).

Transient and frequency domain analysis on these circuits were performed using LTspice IV simulations.

Results

Time Domain Analysis on Plasma and Nuclear Membranes

Comparison Among Different Dielectric Models of the Nucleus

In this section, the comparison among the three different models of the nucleus (see Tables 1 and 2) is reported. In particular, the time courses of the TMP of the nucleus were analyzed in order to better comprehend the influence of the dielectric model on such a quantity for the different types of pulses.

In Fig. 2, the great influence of the choice of the nuclear characteristics is evident. The differences in the time courses are due to the different electric characteristics of the nuclear compartment and depend on the pulse type. In particular, for the model of Hu et al. (2005), the TMP of the nucleus is always lower than that of the other models (with a factor of about 0.3–0.5), due to the higher value of the static permittivity of both the nuclear membrane and the nucleoplasm. The time courses for the model of Merla et al. (2009), Yao et al. (2008) were similar for the longer pulses, while for the shorter ones a significant difference was shown comparing the three models. These results show the great importance in the choice of the dielectric model of the nucleus in order to correctly estimate the nuclear membrane poration.

Dispersive and Nondispersive Dielectric Models of the Nucleus

In this section, a study in the time domain was carried out to demonstrate how different is the TMP response of the nucleus if the dielectric dispersion of the nucleus is included or disregarded. In particular, we applied again the six types of nsPEFs from Table 3. Figure 3 shows, for each type of nsPEF, the TMP on the plasma membrane (dark gray dashed line) and the ones calculated over the nuclear membrane for both the dispersive and the nondispersive nucleus models (values as in Table 1, dark and light gray solid lines in Fig. 3). The results show that the TMP on the plasma membrane always exceeds the poration threshold, while the same extracellular intensities are not able to reach the effect (TMP below the threshold) on the nuclear membrane when the three longest pulses (second row of the Fig. 3) are used. For the shortest signals, on the contrary, the threshold on the nuclear membrane is attained (first row of Fig. 3). As expected, the shorter the nsPEF, the greater the difference between results for the dispersive and nondispersive nucleus models, up to the limit case of ES_nsPEF where the nuclear TMP overcomes the poration threshold of 1 V only when the dispersive model is used. For this last type of nsPEF, in particular, differences between the dispersive and nondispersive models are comparable to the ones obtained with the models from Yao et al. (2008), Hu et al. (2005), demonstrating the fundamental role played by dielectric dispersion for the shorter and faster nsPEFs.

Frequency Domain Analysis on Nuclear Membrane for Dispersive and Nondispersive Models

In order to further study and elucidate the different behaviors of the two circuit topologies, with dispersive

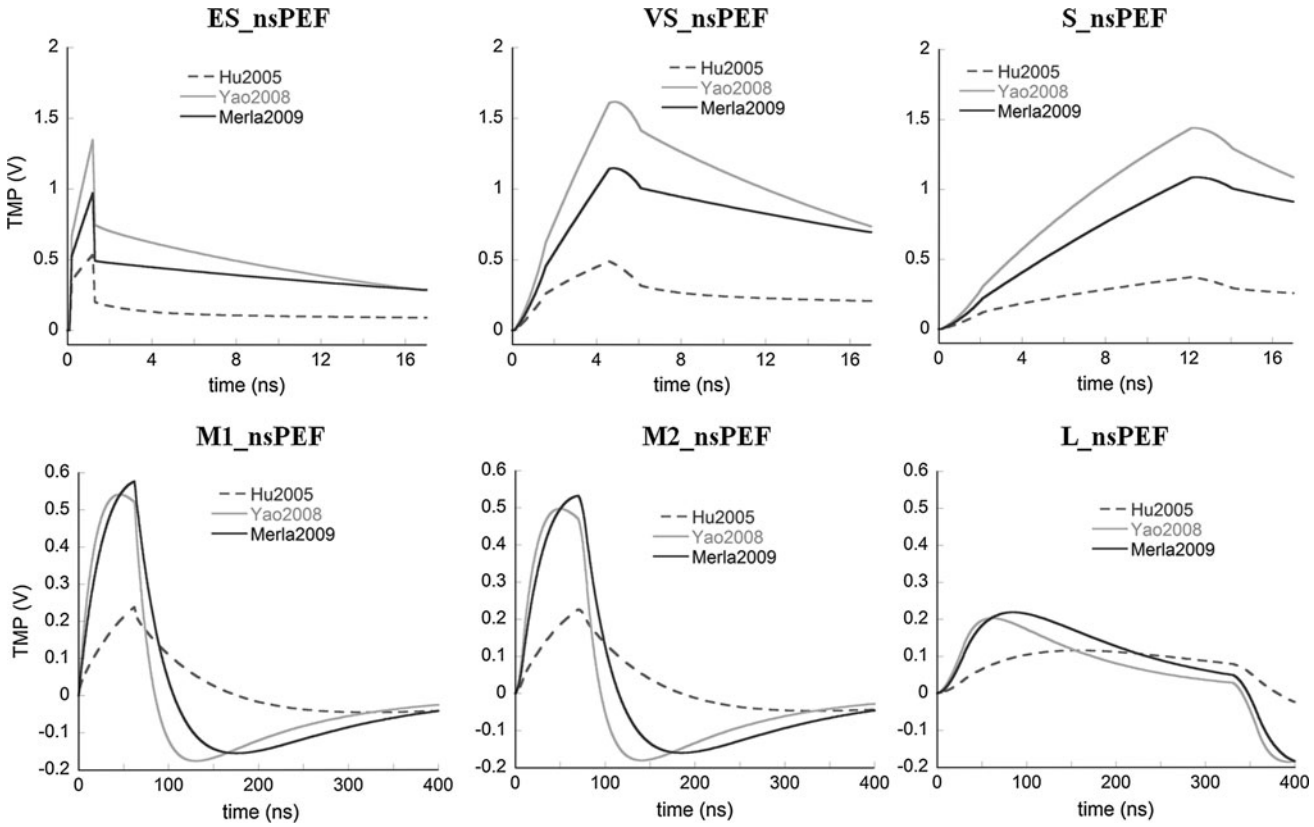

Fig. 2 Time courses of the TMP across the nuclear membrane for different dielectric nucleus models

and nondispersive nuclei, a frequency analysis was carried out. The transfer function of the nuclear membrane—i.e., $H_{nucleus}$ (f) = TMP(f)/V_{in}(f), the ratio of the nuclear TMP over the feeding voltage—was computed. The modulus of the membrane transfer functions is presented in Fig. 4. This figure shows that $H_{nucleus}$(f) curves calculated for the two models start to diverge at around 100 MHz. Before this frequency value the two curves are undistinguishable. These results are in good agreement with the band-pass frequency behaviors of the analytical solutions as reported in Polk and Postov (1995).

In Fig. 4, the normalized amplitude spectra of the shortest (ES_nsPEF, gray dotted line) and of the longest (L_nsPEF, gray light dashed line) input pulses are also reported. From the figure it is evident that the ES_nsPEF spectral components are differently taken into account by the two circuit topologies, while the L_nsPEF ones are considered in the same way. Therefore, one can argue that for the shorter pulses a dispersive nucleus is necessary rather than a nondispersive one. Conversely, for the longer pulses, the dielectric dispersion of the nucleus can be disregarded.

Discussions and Conclusions

In this article, a cell circuit model with nucleus compartment is reported. Three different dielectric nucleus models have been considered; moreover, the dispersive behavior of nuclear compartments has been accounted for. The results have been computed, both in time and in frequency domains, for the six pulse types reported in Table 3.

Results obtained using different dielectric models of the nucleus present in the literature highlighted the importance of the dielectric characteristics of this fundamental organelle. Therefore, the research should focus on the evaluation of the correct parameter values in order to obtain an accurate and predictive microdosimetric model.

Moreover, the responses to the different pulses of the circuit models with dispersive and nondispersive nuclei have been compared. As a first consideration, results in the time domain highlighted a higher intensity of the potentials induced on the nuclear cell membrane and, hence, a deeper electric penetration in the case of the shortest pulses. Indeed, the short pulses permit us to reach the poration threshold in both the plasma and the nuclear membranes, inducing effects even in the interior of the cell. In order to

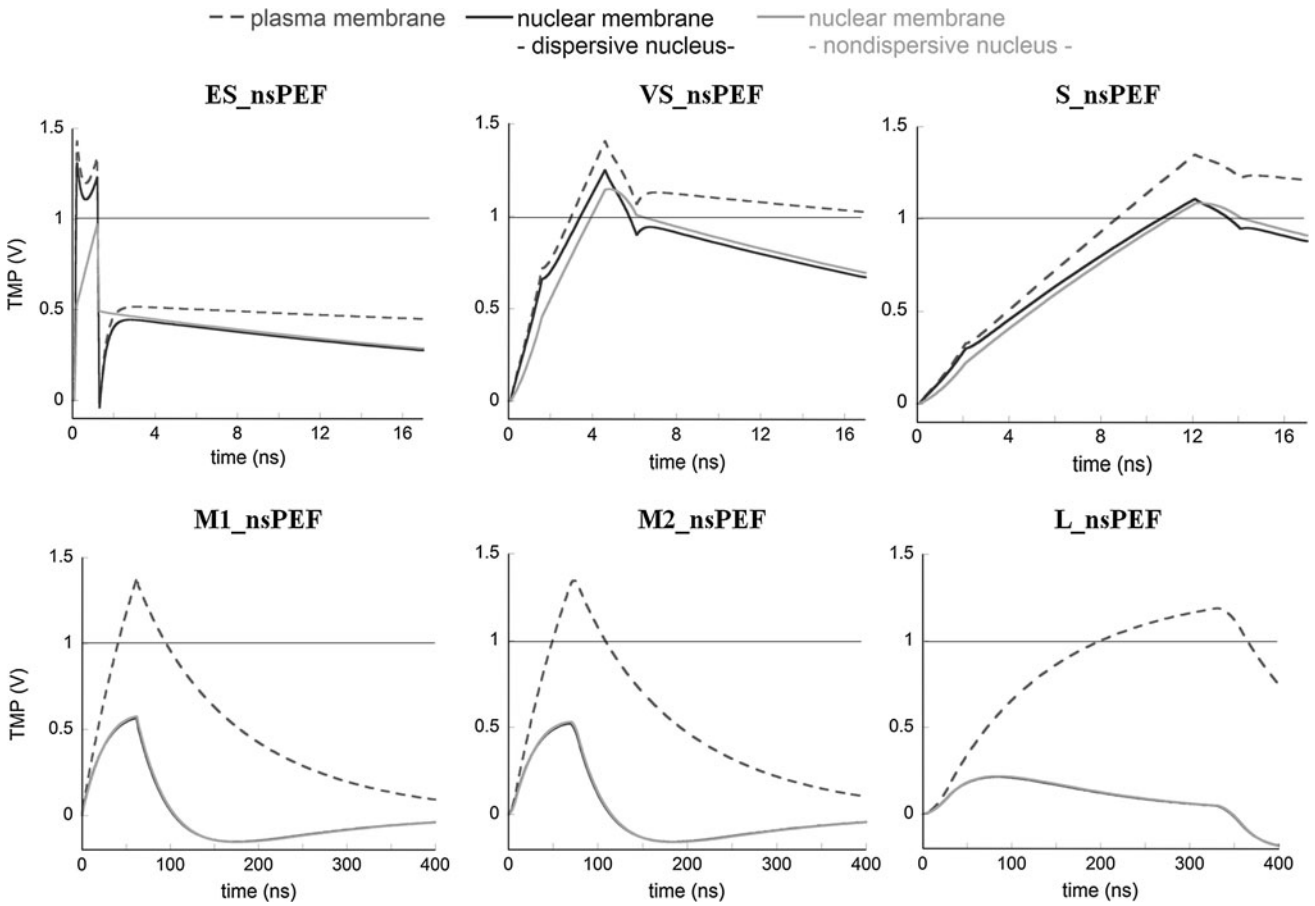

Fig. 3 Plasma and nuclear TMP time courses for dispersive and nondispersive nucleus models

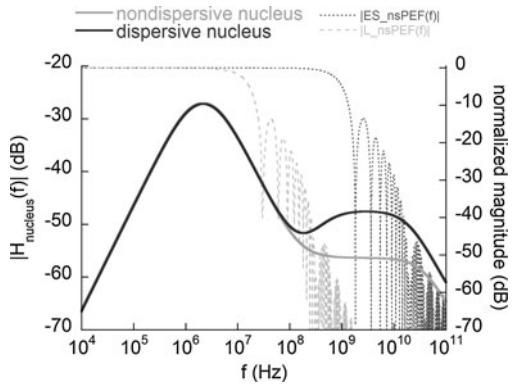

Fig. 4 |H(f)| of the nuclear membrane, $H_{nucleus}(f)$, for circuits with dispersive and nondispersive nuclei

obtain similar effects in the internal compartment with longer pulses, a higher amplitude, than the minimum required to porate the plasma membrane is necessary, with a consequent great increment of the TMP on the plasma membrane.

When the shortest pulses are considered, the TMP on plasma and nuclear membranes is underestimated if dispersion is disregarded. In particular, for the shortest pulse, nuclear dispersion has been revealed as a critical parameter to correctly predict the appearance of effects on the internal membrane. Indeed, disregarding the dielectric dispersion, the TMP on the nuclear membrane remains below the electroporation threshold value (1 V). Such differences can be explained by looking at the circuit responses in the frequency domain. Through the transfer function, $H_{nucleus}(f)$, we observed a different behavior on the nuclear membrane for the two models (dispersive and nondispersive) above 100 MHz, confirming the importance of the dielectric dispersion in the nucleus compartment for the shortest pulses.

Therefore, it is shown how dispersive nucleus models are unavoidable when dealing with the shortest nsPEFs (<10 ns) because of the large spectral contents arriving above 100 MHz, i.e., over the typical relaxation frequencies of the dipolar mechanism of the molecules constituting the nuclear membrane and the subcellular compartments (Polk and Postov 1995).

At the present state of the art, an optimal dielectric model for the nucleus is not available. For this reason, it seems important to develop an accurate model for this fundamental internal structure in order to better comprehend the effects of the nsPEFs.

In conclusion our cell circuit model with nucleus seems helpful to guide the choice of the optimal experimental protocol in terms of appropriate pulse characteristics in order to overcome the TMP threshold not only on the plasma membrane but also on the internal structures such as the nucleus. However, after the onset of the poration processes, more complex models including active elements are necessary (Smith and Weaver 2008).

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