A microdosimetric study applied to ultra short pulsed E field

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Abstract

A number of biological relevant phenomena has been associated to the exposure of cell lines and organs to ultra-short pulsed electric fields (usPEF), suggesting the plasmatic membrane as one of the main interaction targets. In this context a microdosimetric study able to predict E field distribution on the membrane, seems to be particularly interesting. A quasi-static EM solution, both on a three layered spherical cell composed by extra-cellular medium, plasmatic membrane, and cytoplasm, and on a five-layered cell including also the bound water structures, is proposed. By considering three different cell dielectric models, three different cell radius dimensions, three different plasmatic membrane and bound water thicknesses, the role of these important parameters involved is highlighted.

1. Introduction

A fundamental task in approaching the bioelectromagnetic interaction studies is to quantify the electromagnetic (EM) field distribution inside the biological target down to the microscopic cell level (microdosimetry). In fact, only the rigorous knowledge of the field distribution, especially at the microscopic level (single cell and sub-cellular compartments), where the first interaction step seems to occur, allows to elucidate the chain of events that leads to macroscopic (i.e. at tissues, organs or entire body level) observed effects [1]. Recently, relevant effects related to the exposure of different cellular lines and cultures to ultra-short pulsed electric fields (usPEF) have been observed at plasmatic [2] and sub-cellular membrane level [3]. The limited energy provided to cell suspensions by these short pulses eliminates the probability of thermal mechanism [3], nevertheless the mechanisms related to these effects are almost unknown. Particularly, literature data [2] suggests that at cell membrane level the usPEF signals cause the translocation of one of the membrane phospholipids, the phosphatidylserine (PS) to the outer face of the cell membrane. This phenomenon, driven by the trans-membrane potential (TMP), is caused probably by the formation of nano-pores affecting cell functions and signaling, marking the beginning of apoptotic cellular states [2]. Therefore, a microdosimetric analysis, focusing on the EM field distribution induced at membrane level, seems to be appropriate to give an insight in the mechanisms related to pulsed signals effects. In this work the EM analysis has been carried out on a single three-layer spherical cell and an in-depth study of the role of the main parameters involved in the solution of the EM problem, related to both geometrical and dielectric features of the biological structure, has been performed. Moreover, since the presence of hydration shells around the plasmatic membrane has been suggested by a number of theoretical and experimental works [4], a five-layered cell model, taking into account also these structures known as "bound water", has been analyzed. In this way, possible variations induced by the presence of the bound water layers on the membrane field and TMP values have been investigated.

2. Methods

A quasi-static approach has been adopted, since in microdosimetric problems, due to the reduced cell dimensions (up to 50 μm) with respect to the field wavelengths (equal to 3 mm, in air, at 100 GHz), the propagation phenomena of the field can be disregarded. To verify this hypothesis a comparison between a full wave solution on a single multilayered sphere (Mie theory, considering the propagation of the incident EM wave in an equivalent tissue of gray matter (ε_r=40)) and the quasi-static approach (Laplace equation [5]) has been performed. The comparative study evidenced that the quasi-static approximation can be adopted up to 100 GHz for a cell radius of 50 μm (the biggest cell radius) with an error of about 5%, obtained by comparing the field values (real part) of the Mie scattering with the field amplitudes of the Laplace solution [6].

The adopted solution algorithm takes into account the frequency of the incident field through the frequency-dependent dielectric properties of each biological material. Therefore, the dielectric cell model takes into account the dispersive properties of each cell layer (plasmatic membrane, cytoplasm, extra-cellular medium and bound water)

through the Debye equation including the conductivity term (as reported in equation 1), where ε_∞ represents the dielectric constant value at extremely high frequency, ε_s is the static value, ω is the angular frequency, τ is the time constant of the dielectric relaxation process and σ_{dc} is the static conductivity of the material.

$$\varepsilon(\omega) = \varepsilon_\infty + \frac{(\varepsilon_s - \varepsilon_\infty)}{1 + i\omega\tau} - i\frac{\sigma_{dc}}{\varepsilon_0 \omega} \quad (1)$$

The Debye parameters have been extracted through an accurate procedure, that joins dielectric constant measurements of a biological solution (liposomes) with a fitting algorithm based on a proper Effective Medium Theory (EMT) formula (Table 1) [6]. Two other dielectric models, coming from the literature, have been also adopted in the analysis. In a first model, the cell membrane is dielectrically characterized by two relaxation processes, representing respectively the rotational mobility of the phospholipids head group and the orientation of the bound water molecules (Table 1) [7]. In the second one, a non-dispersive behavior for the membrane is proposed, as reported in [8] and in Table 1.
The usPEF signals, having a high spectral content, are often considered as wide band signals. Therefore, a suitable approach is followed. The incident pulsed signal is transformed in the frequency domain by DFT (Discrete Fourier Transform); for each frequency of the signal spectrum the EM solution is calculated, obtaining E field distribution within the cell membrane. Afterwards an inverse DFT is used to transform E field values, obtained by the EM solution in the time domain, in order to re-construct the time behavior of the membrane field. The incident signal adopted for the analysis is trapezoidal shaped one with amplitude of 10^6 V/m and duration of 10 ns. The signal rise time is equal to its fall time with a value of 1 ns. Different cell radius dimensions have been assumed in a plausible range of values, equal to 5, 10 and 25 μm. Furthermore, different thicknesses for the cell membrane (5, 10, 20 nm) and bound water (0.5, 1, and 5 nm) have been also adopted in the analysis.

Table 1 Dielectric properties of the membrane and bound water layers, as proposed from our estimation and from the data reported in [6]-[8].

	Plasmatic membrane						Bound water			
	ε_s	ε_∞		τ (ns)		σ_{dc} (S/m)	ε_s	ε_∞	τ (ps)	σ_{dc} (S/m)
Our [6]	11.9	4.25		1.16		$1.5\text{x}10^{-7}$	34.6	5.5	90.4	0.66
[7]	11.3	-		-		-	80	4.9	398 and 159	1 and 0.001
[8]	5	$\Delta\varepsilon_1$	$\Delta\varepsilon_1$	τ_1	τ_2	$3\text{x}10^{-7}$	-	-	-	-
		2.6	0.84	3	0.46					

3. Results

As a first step, the results (section 3.1) obtained for the three-layered cell are presented. In this case the role of the dielectric model and of the geometrical properties of the cell (radius, and membrane thickness) on the membrane field is discussed. In the subsequent section (3.2), the results obtained for the five-layered sphere are shown. In this case a first comparison with the data coming from the three-layered EM analysis is proposed. Successively, the role of bound water thickness on the membrane field and on the field within the bound water compartment itself is presented and discussed. In both cases results are relative to the maximum membrane field value, where the incident E field direction is parallel to the normal to the cell membrane surface.

3.1 Three layered cell model

The behavior of the E field and of TMP on cell membrane obtained from different dielectric cell models (Table 1, [6]-[8]) is reported Fig.1a. In this case, the cell radius and the membrane thickness have been fixed at a value of 10 μm and 10 nm, respectively. The comparison among the temporal behaviors of the membrane fields (Fig. 1a) demonstrates that frequency-dependent dielectric modeling of the membrane compartment strongly affects these field values. In this case a variation of about 40% between the blue light curve obtained with the dielectric model proposed in [7] and the red curve obtained with the model proposed in [8] is noticeable. This dependence of the E field and of the TMP values on the dielectric model is more appreciable when the rapid time variation of the curves occurs, during the on-set of the incident pulse (up to 12 ns, Fig. 1a). In this case, the high frequency components of the signal prevail and the influence of the dielectric model on the E field data is stronger, as previously evidenced from a microdosimetric analysis on CW sinusoidal signals [6]. On the contrary, the dependence of the field on dielectric model decreases during

the decay of the curves (after 12 ns, Fig.1a), since in this case the low frequency components prevail. At low frequency (up to few MHz), as just evidenced by an earlier EM analysis on CW signals [6], no dependence of the membrane field values on the dielectric model adopted is reported. The dependence of the E field and TMP on the cell radius has also been studied (Fig 1b). This picture reports the comparison among the E field values for three different radii (5, 10, 25 μm, Methods), once the dielectric model has been fixed to the values of [6] (see Table 1). The main differences in the time behavior of the membrane field are observed in the decay parts of the curves (Fig. 1b). This region of the curves corresponds to the low frequency components of the signals, and exactly in this frequency range (up to few MHz) cell size mainly affects the membrane field, as evidenced in [6]. In this case, the variability of the induced membrane E field (Fig. 1b) is of about 40% near 50 ns, increasing for longer time with a maximum value of 80% at 100 ns. The results of E field and TMP within the cell membrane obtained for different membrane thicknesses (with the values reported in Methods) are shown in Fig. 1c. The cell radius adopted is equal to 10 μm, while the dielectric model and the pulse characteristics remain unchanged with respect to the previous ones. In this case the E field values present a more pronounced variability in the decay part of the curves, due to different dimensions of the cell membrane. The TMP, as expected, shows wide variations depending on the membrane thicknesses (about 70%). In fact, when the membrane thickness is higher the membrane capacitance becomes smaller, the time constant associated to the charge of the membrane is reduced, leading to an enhancement of the slope of the TMP curve during the application of the incident pulse, as it is observable from Fig. 1c (blue light line). On the contrary, when the membrane thickness becomes smaller the associated membrane time constant is higher and the slope of the TMP curves is reduced (Fig. 1c red line). Moreover during the incident pulse rise time and fall time (between 0 up to 1ns and between 11 up to 12 ns respectively) the slopes of the TMP vary with the membrane thickness (Fig. 1c). Once the incident signal has run for 12 ns (Fig. 1c) the membrane capacitance continues to discharge through the membrane resistance following its time constant: faster in case of thicker membranes and slower for thinner ones.

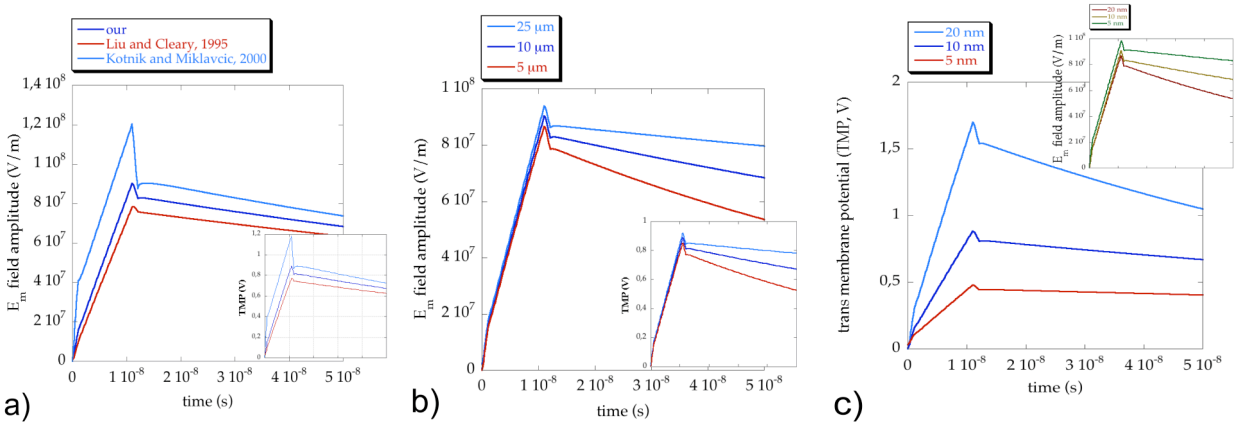

Fig.1 E field and TMP variations induced by the adoption of a) different dielectric models, b) different cell radius dimensions, and c) membrane thicknesses.

3.2 Five layered cell model

A comparison between the TMP in the three-layered cell and in the five-layered configurations, including internal and external bound water layers with a thickness of 1 nm, is proposed in Fig. 2a. It is possible to observe that the introduction of the bound water influences the value of the TMP. Therefore, such a structure has to be accurately studied to obtain a rigorous quantification of the field.

Furthermore, the influence of different bound water thicknesses on both the TMP values and on the potential across the bound water layer has been investigated. While no variations in the TMP are induced by different bound water thicknesses (values reported in Methods), variations of about 80% in the potential across the bound water layers are evidenced (Fig. 2b). In this last case a bipolar behavior of the potential is also evident.

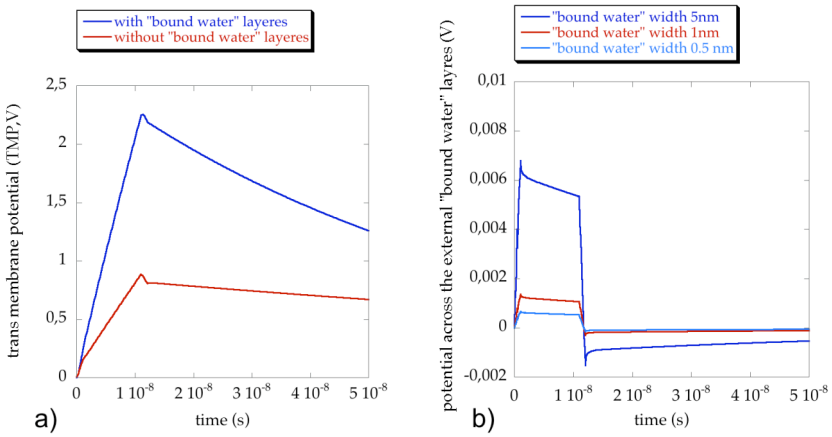

Fig. 2 a) A comparison between the TMP values obtained for a three and five-layered cell configuration; b) Potential across the bound water layer obtained for different thicknesses of the bound water environment.

4 Discussion and Conclusions

A complete microdosimetric analysis focusing on usPEF fields has been proposed. An analysis adopting the Laplace equation on a spherical multilayered cell has been realized and properly adapted to take into account this kind of wideband signals. The analysis has evidenced the role of different parameters involved in the problem, as the dielectric model, the cell size, and the membrane thickness. From this microdosimetric study different noticeable points have emerged. First, the introduction of the Debye equation in the characterization of the cell compartements is recommended especially for an accurate quantification of the E field or TMP peak values, since these quantities are mainly involved in the poration dynamics of the plasmatic membrane [2]. Second, to accurately describe the field or TMP decay, a realistic cell dimension must be taken into account, since in this case the low frequency components of the signal prevail. Third, the presence of the bound water environment strongly affects the membrane field and the TMP values, being therefore of relevance for accurate microdosimetric results. Moreover, different bound water thicknesses have no influence neither on the field, nor on the potential values at membrane level, while these differences affect the potential across the bound water layer itself, suggesting that a proper quantification of the thickness of the bound water layers can be relevant only to know the field distribution in this last environment. From all these considerations the performed EM analysis seems to be a useful support in the comprehension of the action mechanisms of the usPEF at the cellular level, resulting extremely relevant for a number of possible emerging treatments as cancer or gene therapy.

7. References

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