Modelling of the temperature gradient across biological cell membranes stressed with pulsed electric fields

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Abstract: ***Membranes of microorganisms stressed with pulsed electric fields (PEF) of sufficient intensity and duration can be permanently damaged by irreversible electroporation. Such PEF-induced damage of a biological cell membrane can lead to the death of the microorganism; this process facilitates practical applications of PEF for microbial inactivation in liquids and lysis. PEF treatment is considered a "non-thermal" inactivation process: typically the global temperature of liquid samples treated with impulsive electric fields remains below the thermal inactivation threshold. However, intense electric fields may result in the development of local temperature gradients across biological membranes. Thus, it is important to investigate these local heating effects for further understanding and optimisation of PEF treatment of microorganisms. Pore formation also happens in biological membranes during PEF treatment, with simulation results showing that local heating also exists in these pores. These heating effects may help enhance the process of the formation of pores during PEF treatment. This local heating in a pore is shown to be influenced by the position and dimension of the pore.***

I. Introduction

Pulsed Electric Field (PEF) treatment is one of the practical applications of pulsed power technology. PEF treatment has demonstrated excellent potential for the inactivation of microorganisms, and can be used in the food industry, for biomedical and other applications including cell lysis. Multiple research papers on PEF inactivation of microorganisms have been published in recent years, including [1]-[6]. Electroporation is generally considered the main process occurring in the biological cells during PEF treatment, [1]. Pores in biological membranes can be created when cells are exposed to an external electric field; such pores can be either reversible or irreversible, depending on the electric field strength and the PEF treatment time. The formation of irreversible pores can lead to cell lysis. PEF treatment is generally considered a non-thermal process on the basis that there is no significant temperature increase in the bulk liquid stressed with the impulsive electric fields, [2].

However, local heating effects do occur, which may cause structural damage to the biological membranes or cell organelles, and thermal deformation of the membranes may also stimulate the formation of pores or their structural damage. Furthermore, as pores may be filled with conductive liquid (cytoplasm or/and surrounding liquid), Joule heating may lead to increase of the temperature inside and around the pore. This local heating process may stimulate the expansion of the pore and facilitate electroporation during the PEF treatment. For example, in [3] it was shown that a single ns HV pulse can lead to an increase in temperature over small section of the biological membrane, which can facilitate the pore formation process. It was suggested that the decrease in viscosity of the membrane with temperature could be the main cause of acceleration of the electroporation process, [4]. Therefore, the local heating effects in and around the pores and microbial cell membrane in general may also play significant roles in the PEF inactivation and lysis processes. Thus, it is necessary to investigate the interplay between the local heating process in biological cell membranes and the pores, and the intensity, distribution and duration of the applied electric field. The electric field distribution in the cell membrane during PEF treatment is non-uniform, so the position of pores may also have an influence on the thermal effects in the pores. The formation of pores is generally a continuous process; pore expansion is also observed during the PEF treatment. The diameters of the pores may also have an influence on local heating effects. Investigation of local heating in and around the generated pores may help to further understand the electroporation process and can contribute to optimisation of the PEF process and its practical applications.

To investigate this, a single cell model with parameters based on real microorganisms was developed using COMSOL Multiphysics software. A DC voltage was applied to generate a uniform field of magnitude 25 kV/cm between two electrodes in this model, and the pulse duration in the model was varied from 1 μs to 25 μs. The electric field distribution in the membrane and local heating effects were investigated. In addition, a model pore was introduced into the membrane to simulate the electroporation process.

II. Single Cell Models and Simulation Results

A. Single cell model with COMSOL Multiphysics software

The single cell model includes 3 main elements: the PEF treatment electrodes, the single cell microorganism, and the external fluid surrounding the cell. The single biological cell is modelled as a spherical structure which consists of a cell wall, cell membrane and internal cytoplasm.

The basic structure of the single cell model is shown in **Figure 1**. The surrounding fluid represents a water-based

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growth medium whose conductivity is lower than that of the cytoplasm. The cell wall surrounds the biological membrane made up of a lipid-bilayer and proteins which keeps the cytoplasm in a relative stable environment. From an electrical perspective, a biological membrane can be regarded as an insulator with low electrical conductivity. The cytoplasm is the main arena for cellular activities; chemical reactions take place in the cytoplasm within the cell therefore there are ions within the cell, and the conductivity of the cytoplasm is usually higher than that of other components in this model. The parameters used for the single cell model are shown in Table 1.

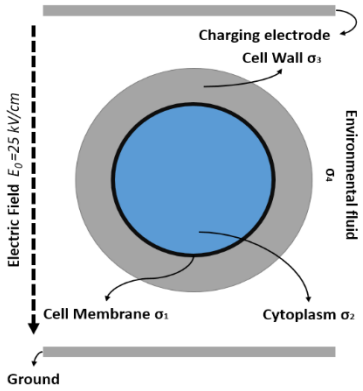


Figure 1. Structure of single cell model with all elements

TABLE I
BASIC PARAMETERS OF SINGLE CELL MODEL

Parameters	Value
Relative permittivity of cytoplasm	80
Relative permittivity of membrane	2
Relative permittivity of environmental fluid and cell wall	80
Conductivity of cytoplasm	1.2 S/m
Conductivity of cell membrane	10^{-7} S/m
Conductivity of environmental liquid and cell wall	0.1 S/m
Thickness of membrane	5 nm
Diameter of cytoplasm	5 µm
Width of treatment region	10 µm

The steady-state electrical field distribution in the spherical cell membrane is considered to follow the Schwan Equation, [5], shown as equation (1) here,

$$\Delta\varphi = 1.5 \cdot E \cdot R \cdot cos\,(\theta) \qquad (1)$$

where $\Delta\varphi$ is the induced membrane potential, E is the external electric field, R is the radius of the membrane and θ is the angle between the electric field direction and the vector normal to the surface of the cell. It can be seen from equation (1) that the induced membrane potential is proportional to the magnitude of the external electric field strength and to the radius of the cell membrane, which means that a higher trans-membrane potential will be induced on the membrane for larger cells. The induced membrane potential is also a function of the angle θ. Therefore, a maximum membrane potential will be developed at the 'poles' of the membrane where $\cos(\theta) = \pm 1$, [6]. The electric field strength therefore will be at maximum at the poles, and minimum at the equator. **Figure 2** shows the nominal electric field strength in a quarter of dielectric membrane as a function of positions, from pole to equator, the trendency follows the Schwan Equation The electric field strengh in the external fluid, E_0, is 25 kV/cm. Although the maximum field in Figure 2 may exceed the nominal breakdown field of the cell membrane, this model is used to simulate the heating effects in the pore(s) at the field strenght, E_0, which is typical for PEF treatment, [6].

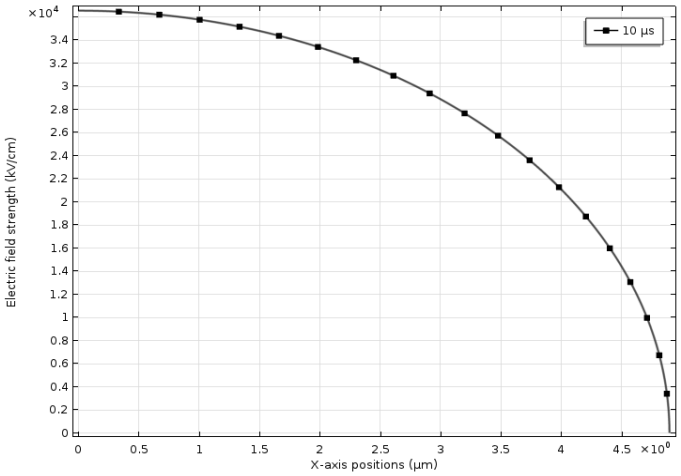


Figure 2. Maximum electric field strength in the membrane as a function of X-axis at 10 µs.

In order to investigate the local heating effects, thermal parameters were introduced to the model, and Table 2 lists the thermal parameters used.

Parameters	Environmental fluid	Cell membrane	Cytoplasm
Heat capacity at constant pressure J kg^{-1} K^{-1}	4181.3	3000	4181.3
Thermal conductivity J K^{-1} m^{-1}	0.61	0.568	0.61
Density kg m^{-3}	998.2	1100	998.2

TABLE 2
PARAMETERS USED FOR THERMAL ANALYSIS OF THE SINGLE CELL MODEL

As the cell wall is a porous structure which is filled with the external liquid, the thermal parameters of the wall are considered to be the same as the parameters of the external liquid, so the wall was excluded from the current analysis. In this model the cell membrane is effectively surrounded by the environmental fluid.

In [7] the local heating effects during PEF treatment have been modelled: the initial temperature of the environmental fluid and biological membrane components was 20°C. The simulation shows that the local temperature in the environmental fluid can reach more than 25°C within 2 µs due to Joule heating. However,

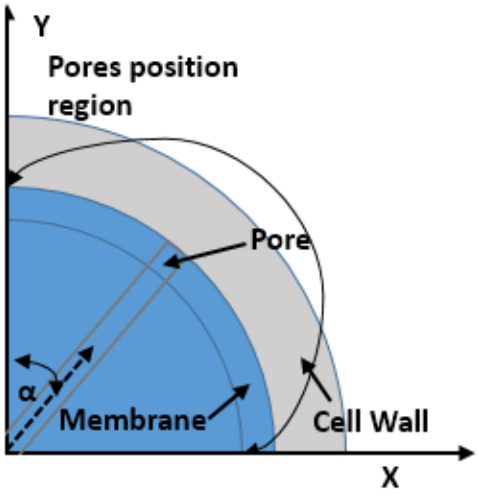
there is no increase in temperature in the cytoplasm. There is no electrical conduction in the cytoplasm after completion of the field relaxation process when the local field is the system becomes the steady-state field, [8]. The local heating effects observed, may help to stimulate the formation of pores and membrane structural deformation and damage.

B. Single cell model with pore introduced

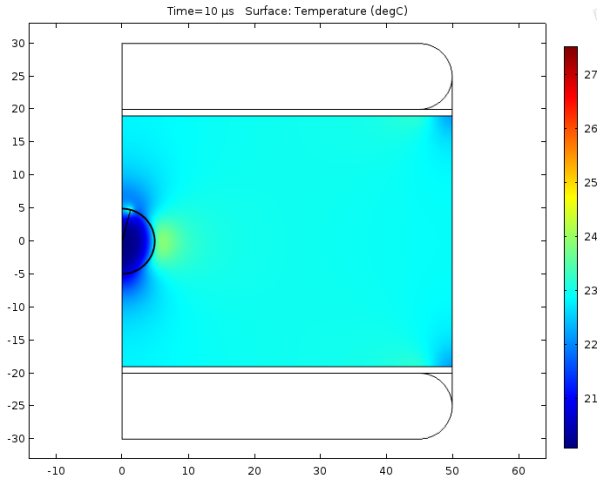
To investigate the local heating effects in a penetrated membrane, a pore was introduced introduced into the model membrane, **Figure 3** (a). The pore can be regarded as having penetrated the biological membrane. There is an angle, α, between the central axis of the pore and the Y axis of the model. The angle α represents the position of the pore introduced in the membrane from 0 ° (Y axis) to 90 ° (X axis). The conductivity of the fluid in the pore was set to be equal to that of the surrounding fluid, 0.1 S/m, and the relative permittivity was 80. The thermal parameters were set to be the same as environmental fluid. In order to simplify the model and the process of investigation of the membrane behaviour, the influence of the cell wall was eliminated by setting its electrical parameters to be the same as surrounding fluid. **Figure 3** (b) and (c) shows the temperature distribution of the single cell model wth pore included.

From the temeprature distribution graph, (b) and (c) of **Figure 5**, the existance of a pore does not influence the local heating effects in the surrounding fluid. The local heating effect in the pore is significant with temperature increasing to ~27 °C in 10 µs when α =15°, and the pore diameter, *d*=1 nm.

Figure 4 shows the effects of pore diameters and positions on the electric field and local heating effects. When α increases, the electric field strength decreases as the distribution of electric field in the membrane follows the Schwan Equation (1). The pore diamater has significant impact on Joule heating in the pore, with more Joule heating occuring as the pore diameter increases. Therefore, the formation of pores and local heating effects in these pores may make a greater contribution to the electoperation process during PEF treatment.



(a)



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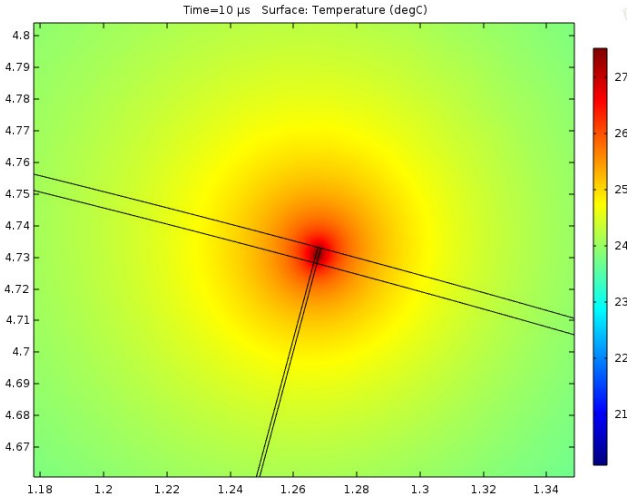
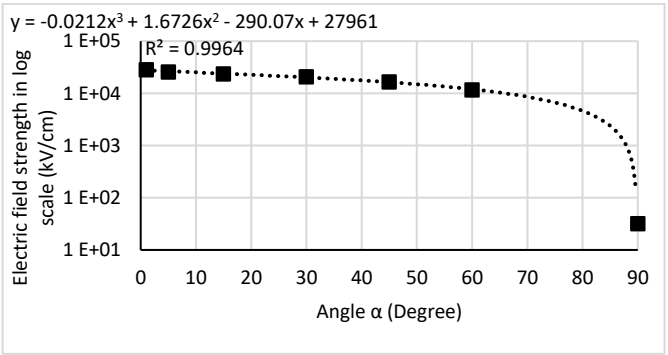
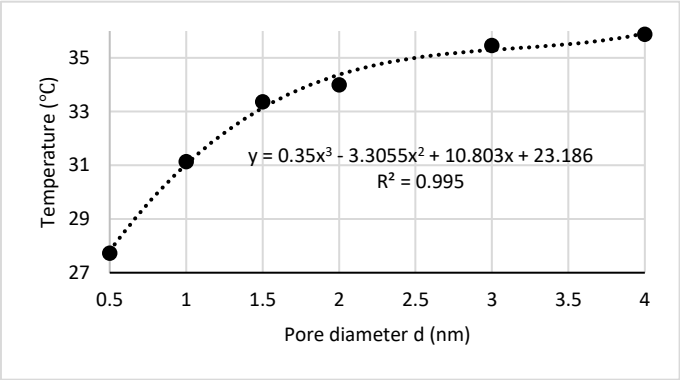


(c)

Figure 3. (a) Pore introduced to the model membrane; (b) Temperature distribution at 10 µs with pore when α =15°, pore diameter d=1 nm, (c) Zoomed in view of (b). Axis dimensions in µm.



(a)



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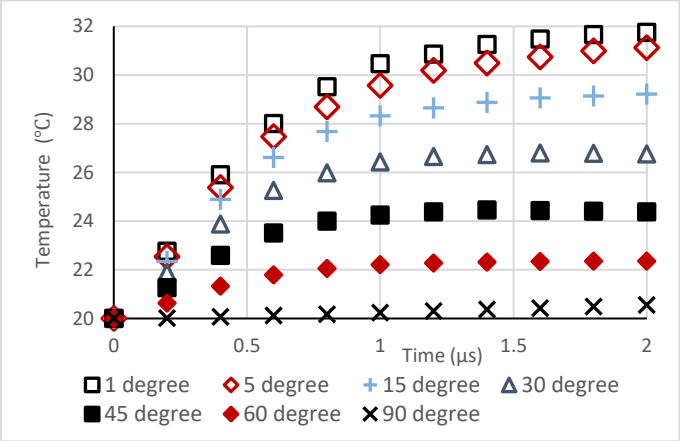

(c)

Figure 4. Simulation results of the single cell model with the pore in the membrane: (a) Maximum electric field in the pore as a function of α for t= 25 μs, (b) Maximum temperature as a function of the pore diameter at t= 25 μs and α=5° , (c) Maximum temperature in the pore as a function of time for different α, d=1 nm.

III. DISCUSSION AND CONCLUSION

The single spherical cell model was developed in the present paper for the analysis of the electric field distribution in the membrane, the cytoplasm and the surrounding fluid during PEF treatment of microorganisms. It was shown that maximum electric field strength in the membrane is a non-linear function of duration of field, and for the present model this field reaches its saturation value after ~1.5 μs. It was also shown that the electric field strength is at maximum at the poles of the spherical membrane, and minimum at its equator. Using the model presented in this paper, it was shown that the inclusion of a pore in the membrane does not have a significant effect on the local temperature in the surrounding fluid. The local heating of the pore can be notable, given that the pore is filled with conductive fluid, and becomes more pronounced when the pore is located closer to the pole of the cell due to the higher electric field strength. As the local heating effects in the pore and surrounding fluid are not negligible, the heat generated on a time scale of a several microseconds is likely to affect and enhance the electroporation process.

According to the simulation results, pores with large diameters reach higher increases in local temperature than those with smaller diameters during the same treatment time. Once a pore is formed, the local thermal effect in the pore may be able to enhance the expansion of the pore and generate more heat, and such processes may accelerate the lysis of cells. The local increases in temperature in the pores and the surrounding fluid could contribute to damage to the structure of biological cells during PEF treatment.

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