Models of Irreversible Electroporation: A Review and Initial Model Implementation

Tihomir Georgiev [1], Kristina Bliznakova [1], Zhivko Bliznakov [1], Georgi Todorov [2,3]

[1] *Department of Medical Equipment, Electronic and Information Technologies, Medical University "Prof. Dr Paraskev Stoyanov" – Varna, Bulgaria*
[2] *E.S. Interventional radiology; Diagnostic imaging, radiotherapy and interventional radiology dept.; Faculty of Medicine; Medical University "Prof. Dr Paraskev Stoyanov" – Varna, Bulgaria*
[3] *Interventional radiology centre, Imaging diagnostics clinic, "St. Marina" University Hospital – Varna, Medical University of Varna, Bulgaria*

Abstract **– Cancer may affect any person. Amongst possible techniques to treat some cancers is ablation, through the use of the irreversible electroporation (IRE) as one of the methods. Medical University of Varna is implementing a project on irreversible electroporation aiming to develop a hardware prototype. The first step is review of existing models and the development of an own model, which will be used in the device prototyping. To find the existing models, we reviewed articles related to electroporation models. Based on the literature review, we developed own model, where we used open-source software OpenEP and Embarcadero C++. The review of the available literature publications indicates that initial models employed a simplified cell structure. Despite this simplicity, these models effectively reflected important characteristics of the cell behaviour and the interdependencies between field features and cells. Recent investigations created better models and optimized parameters for specific cases. The review models were used in the development of dedicated software application for simulation, visualization, and educational purposes in the field of IRE. Developed software application allows investigation of various IRE protocols. Experimental investigation of implemented models and software is undergoing. The software will allow further advancement in our capacity to explore and comprehend cellular dynamics.**

Keywords **– Electroporation models, irreversible electroporation models, software application, pore density.**

DOI: 10.18421/TEM131-54
https://doi.org/10.18421/TEM131-54

Corresponding author: Tihomir Georgiev,
Department of Medical Equipment, Electronic and Information Technologies, Medical University "Prof. Dr Paraskev Stoyanov" – Varna, Bulgaria
Email: t.georgiev@mu-varna.bg

Received: 20 October 2023.
Revised: 02 February 2024.
Accepted: 07 February 2024.
Published: 27 February 2024.

1. Introduction

Cancer affects everyone, regardless of age, gender, or social status, and is a huge burden on patients, families, and society at large. If no further action is taken, the number of people diagnosed with cancer each year in Europe will increase from the current 3.5 million to more than 4.3 million by 2035 [1].

Electric pulses that increase permeability of the cells via creation of temporary pores can be used with chemotherapy in order to treat cancer. Electric gene transfer is considered as a possible treatment as well. Creation of such short-living pores is called electroporation [2], [3], [4].

There are some other methods – various temperature-based ablation techniques aimed at cell death – radiofrequency and microwave ablation, cryoablation, high-intensity focused ultrasound. However, due to their thermal nature these techniques damage also the non-tumour cells and therefore, have their own limitations depending on tumour position in the body [3], [4].

Electrochemotherapy and ablation techniques, however, are usually considered for patients with non-resectable tumours to whom surgical treatment cannot be applied [2], [4].

Contrary to electroporation when pores are only temporary, there is a method called irreversible electroporation (IRE), where pores created using this technique are permanent, and the technique is not temperature related.

Therefore, the IRE technique is considered a prospective alternative of thermal ablation techniques [3], [4]. There are reports that IRE can be used for treatment of cancers, among them liver, [3], [4], [5], [6], pancreas [5], [6] and prostate [6], [7], but also kidney [8], and breast [9].

The application of this technique requires a lot of optimization work, in terms of electric field intensity, design, and location of electrodes depending on the tumour formation, pulse duration, which parameters are not optimized depending on the cancer. Moreover, IRE for breast tumours have not yet been applied in practice, and research is scarce.

Medical University of Varna is implementing the IRE project, funded by the Bulgarian National Science Fund, aiming to develop and test a novel hardware prototype of the IRE technique. The prototype will be evaluated on printed samples from tumour cell lines. A particular application is the IRE of the breast lesions. The first step in this project is the accomplishment of a review on available IRE models and development of a model for IRE that will be used in IRE prototyping.

2. Methodology

For the literature review, we performed search in PubMed database with the following keywords and phrases: "electroporation", "electroporation model" and "irreversible electroporation model". Based on the articles, the theory, calculations and proposed models for electroporation and IRE were summarised.

An own computational model was developed based on the summarised literature review in the next section. To generate pores, we followed the methodology outlined by DeBruin and Krassowska [10]. The key steps within a given timeframe include: (i) computing the new quantity and total number of pores, (ii) calculating the pores within a specified cell area, (iii) determining the total electroporation current through all pores, (iv) updating the transmembrane voltage (Vm). All this is implemented under Embarcadero C++ with the use of OpenGL for visualising the result of the electroporation. Moreover, to deploy various electrode configurations, we utilized the open-source software OpenEP [11] for visualizing the temperature distribution in the simulated tissue under a specified applied voltage.

3. Results and Discussion

Simplified models

Application of electric field with enough strength on tissues causes creation of pores and changes in conductivity and permeability, a process called electroporation (EP) [12], [13], [14], [15], [16], [17], [18].

Pulses for electroporation are with strength of hundreds of V/cm and in microseconds and milliseconds range [13] or up to 100 μs and strength of 1000 V/cm [17], while those for IRE have strength typically 1500 V/cm, but may be between 500 and 2500 V/cm [17], [19], in general.

Several studies investigated the effect of pulsed electric field on a cell by imposing certain simplifications such as a single cell with spherical shape and a thin membrane, no organelles inside, resistivity of the membrane to be relatively higher than the surrounding media. These assumptions lead to Equation (1), where Vm is the transmembrane voltage, f is a function depending on cell parameters, E is the field strength, R is the radius of the cell, θ is the angle of the field direction and τ is the time constant [20], [21], [22], [23], [24].

$$\mathrm{Vm(t)} = f\mathrm{ERcos}\theta\left[1 - exp\left(\frac{-t}{\tau}\right)\right] \quad (1)$$

Equation (1) is transformed in the form of Equation (2) for pulses much longer that the time constant and f is considered equal to 1.5 under the assumption that the resistivity of the membrane is higher than those of the surrounding media [10], [20], [21], [22], [23], [24].

$$\mathrm{Vm(t)} = f\mathrm{ERcos}\theta \quad (2)$$

Model of Kotnik et al.

Kotnik *et al.* [21] reported analytically calculation of function f, represented by Eq. 3 and the time constant in Eq. 4,

$$f = \frac{3\sigma_0[3dR^2\sigma_i + (3d^2R - d^3)(\sigma_m - \sigma_i)]}{2R^3(\sigma_m - 2\sigma_o)(\sigma_m + 0.5\sigma_i) - 2(R-d)^3(\sigma_o - \sigma_m)(\sigma_i - \sigma_m)} \quad (3)$$

$$\tau = \frac{RC_m}{\frac{2\sigma_o\sigma_i}{2\sigma_o + \sigma_i} + \frac{R}{d}\sigma_m} \quad (4)$$

where R is the cell radius, Cm is the membrane capacitance, d is the membrane thickness, and σ_i, σ_m and σ_o are cytoplasmic, membrane and extracellular conductivities.

Results reported by Kotnik *et al.* [21] with their models showed significant influence of the extracellular conductivity on function f and τ, but only in case extracellular conductivity values used for modelling were much lower than physiological ones, and Eq. 1 was then applicable, while with typical physiological values the results were close to Eq. 2 [21]. The same group developed a method and derived equations for different type of pulse shapes [22] and for transmembrane voltage on prolate and oblate spheroids [23].

While internal structures of the cell are almost unaffected with the electroporation in microsecond range, it is not valid with pulses in the nanosecond range (nsPEF) and strength up to hundreds of thousands of volts per centimetre - the internal structures of the cells can be affected more than the cell membranes [13]. A model was developed that included a single organelle inside the cell and calculated intracellular membrane voltage induced by sinusoidal and trapezoidal shaped pulses. Results reported by the authors showed that organelle transmembrane voltage may become greater than the cell transmembrane voltage under some requirements on conductivities and with pulses in the nanoseconds range [13].

Model of DeBruin and Krassowska

This model was performed to investigate the cell behaviour related to electroporation [10]. Specifically, it determined the resistance-capacitance behaviour of the cell observed on the cell equator, while near the poles the voltage rises more rapidly and also pore density steeply rises but was also significantly higher than on the equator. According to their model, the pore density at any time can be calculated as per Equation 5 [10].:

$$\frac{dN}{dt} = \alpha e^{\left(V_m/V_{ep}\right)^2\left(1-\frac{N}{N_0}e^{-q\left(V_m/V_{ep}\right)^2}\right)} \quad (5)$$

The current through the pores is calculated by Equation 6, Equation 7, and Equation 8 [10]:

$$I_{ep} = N i_{ep} \quad (6)$$

$$i_{ep} = \frac{\pi r_m^2 \sigma v_m RT \frac{e^{v_m}-1}{\frac{w_0 e^{w_0-nv_m}-nv_m}{w_0-nv_m} e^{v_m} - \frac{w_0 e^{w_0+nv_m}+nv_m}{w_0+nv_m}}}{Fh} \quad (7)$$

$$v_m = V_m \frac{F}{RT} \quad (8)$$

In their equations, N is the pore density, N_0 is the pore density when the transmembrane potential is 0, i_{ep} is the current through a single pore, r_m and V_m are the pore radius and transmembrane potential, respectively, h is the membrane thickness, σ is the conductivity, w_0 is the energy barrier, n is the pore entrance length, q and α are constants calculated by the authors, V_{ep} is electroporation characteristic voltage also provided by the authors, and R, F and T are universal gas constant, Faraday's constant and the temperature [10].

Results, reported by the authors, showed that there are some differences in the potentials and pores density between the two poles due to the cell rest potential and these differences were usually small except in certain specific conditions about magnitude of the applied voltage and its application time. It was also shown that despite the fact that electric discharge happens with microseconds after end of the electric pulse, the cell resealing time was about 20 seconds. Results from modelling were similar to experimental ones [10]. Further research using the same model [25] determined the small pores with radii close to 1 nm vastly outnumbered the larger pores. Though, small pores had significantly less total area, and played a lesser role in the membrane conductance. However, on the poles these small pores were with more significant part of the total area and more than half of the conductance. Further on, electric field above certain value did not influence the number of the large pores, but only increased the number of the small pores and their contribution to the membrane conductance. The authors determined that the large pores turned into small ones very fast after the end of the pulse.

Model of Vajrala

The cell however has other structures inside and the electric field effects on them should not be omitted. A study [24] modelled a cell and a mitochondrion inside it, and separately only a mitochondrion. The authors concluded that especially for low frequencies the induced potentials on the outer mitochondria membrane for a mitochondrion only model were different than those for a cell and a mitochondrion model, due to the cell membrane screening effect, as well as, the inner mitochondrial membrane induced potential greatly depended on mitochondrial matrix conductivity.

Model of Deng

Different behaviour of the cells' membranes in the nanosecond range was described by Deng *et al.* [12] in an experiment that used dyed Jurkat cells to observe uptake, i.e. membrane permeability under pulses, both longer and shorter than a microsecond. The results showed that with 60 ns pulses the uptake was delayed and several explanations had been considered. Frey *at al.* [26] also performed research using dyed Jurkat cells. The authors observed decreased fluorescence using shorter pulses and suggested the nanopores that were created, were too small. Then, the dyed molecules could not pass through these small openings.

Model of Gowrishankar and Weaver

Gowrishankar and Weaver [27] described a method for creation of models based on equivalent electric circuits and transport lattice. The authors represented the electrolyte by a resistor and a capacitor. The membrane was represented by three components – two membranes and a component consisting of a current source, resistors, and a capacitor. The authors modelled a cube with connections between nodes the electric subcircuits of either the membrane or electrolyte and then applied Kirchhoff's laws. Results, reported by the authors, were very close to the results, based on the traditional analytical method, while their method does not require the specific shape of the cells neither requires application of same properties for different regions. Further development of the model to include a structure inside each cell [28] and using it for the so-called supra EP research included electrolyte and three different types of membranes subcircuits. The authors then compare effects from more traditionally used up to 2 kV / cm, 100 μs pulses and 10-80 kV / cm, 300 ns pulses. The weaker pulse electroporated the plasma membranes but also small part of the nuclear membranes. The stronger pulses electroporated almost all membranes. The authors also determined that with supra-EP pulses the pore density against time is more spatially homogenic and pore density against voltage is almost equal amongst the three types of membranes, contrary to results with traditional electroporation pulses.

Model of Corovic

Mathematical models for single and composite tissue were created by Corovic *et al*. [14] based on results of in vivo experiments. The conductivity is modelled as a function of the strength applied electric field and compared R^2 between in vivo results and models with constant and non-constant conductivity. They reported R^2 values with non-constant conductivity modelled for rat liver by both, inverse analysis and nonlinear parametric analysis, are very close to each other and much larger than values with constant conductivity, while models based on rabbit liver tissues revealed some difference between inverse analysis, nonlinear parametric analysis, and sequential analysis and particular advantages of each of them. The results for composite tissue indicated that different types of tumours are better fit by different types of conductivity models, and that the higher conductivity of the tumour, the higher the applied voltage has to be [14].

There are many research studies using different cell lines, in order to investigate and determine different electroporation parameters.

Developed Software Applications

Several software applications have been developed for simulation of electroporation. Application ApiVizTEP that simulated electric field distribution depending on electrode positions, electroporation parameters, tissue area, etc. was built with educational purposes [29]. There is a free web application EView. Input parameters for the simulation are field strength, electrode configurations, and the types of the tissues. The output is the electric field. The application has been validated by comparison with finite element analysis platform [30]. Another free application OpenEP was built to simulate EP treatment depending on electrodes and their position, electric pulses parameters, etc., and was validated by comparison with literature data with results from theory and experiments [11].

These applications, however, are not compatible with models developed by our group, which imposed the need for our own developments.

Our Contribution - Developed Software

We developed a software application, consisting of two parts: (a) graphical user interface for controlling the parameters of the applied voltage, pulses, electrode configuration, as well as tissue characteristics; (b) subroutines, based on the open-source software OpenEP [11]. It was adapted to run under Embarcadero C++, with optimised subroutines running in parallel, using a shared memory model. All simulations run in three-dimensional domain.

To test the application, we modelled the tissue as a parallelepiped with dimensions 20 mm x 20 mm x 10 mm, made of breast tissue. Two electrodes of type "needle" are placed in a distance of 5 mm. We applied a voltage of 250 V/cm which resulted in the electric field strength of 125 V. Figure 1 shows the developed model and simulations under the conditions, specified in the figure, while Figure 2 shows the result of the simulated electroporation. This simulation shows the distribution of temperature, where the maximum value is 52.85 °C and the minimum is 36.85 °C. Further on, the distribution of the created pores in a single cell is calculated and visualised, as shown in Figure 3 (right image), based on the model of DeBruin [10]: Equation 5 which solution presents the created new pore density.

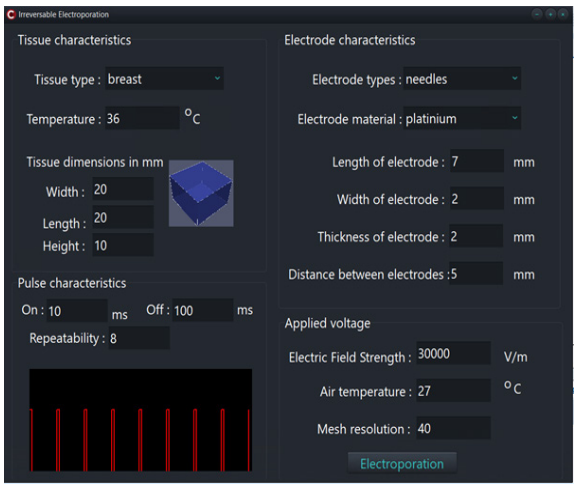

Figure 1. GUI of the IRE simulator, developed at the Medical University of Varna.

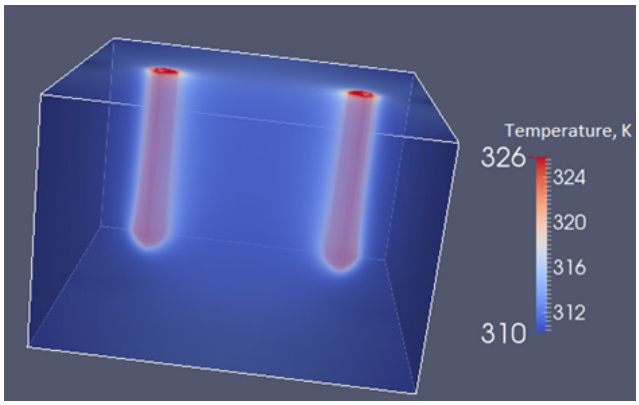

Figure 2. Simulated electroporation in breast tissue. Distribution of the temperature is demonstrated.

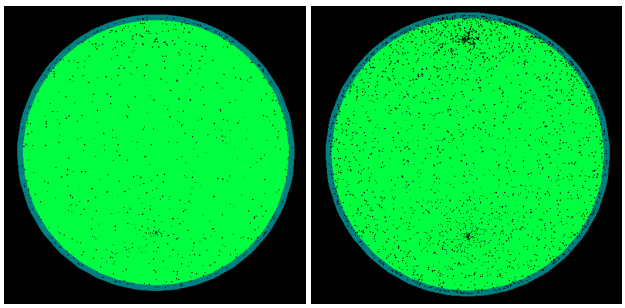

Figure 3. Distribution of pores in a cell with radius 50 µm for 25 kV/m and 40 kV/m electrical fields, each delivered through 1-ms pulses over a cumulative duration of 10 ms.

For comparison purposes, the left and right images in Figure 3 show the distribution of the created pores on a single cell for electric field intensities: 25 kV/m and 40 kV/m, delivered through 1-ms pulses over a cumulative duration of 10 ms. The applied equations are Equations 1 to 8. The pore densities (number of pores per m^2), simulated under the two different electric field intensities (25 kV/m and 40 kV/m) with 1-ms pulses over a total duration of 10 ms, are: $2.6*10^{10}$ and $1.7*10^{11}$, respectively.

4. Conclusion

This paper reviewed the historical development and creation of more complex models of the process of irreversible electroporation. Based on this review dedicated software was developed, which is the base for the development of electroporation and irreversible electroporation in heterogeneous tissues. The simulation is based on the OpenEP package, with GUI that provides enhanced options for model simulations. In addition, a module for calculation of the pore density was developed and visualisation is provided. This in-house software tool allows the investigation of various protocols for electroporation and irreversible electroporation before their clinical use. Experimental validation of developed models and protocols is undergoing.

Acknowledgements

This work is supported by the Bulgarian National Science Fund under grant agreement No. KP-06-N53/12.

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