Modeling and Simulation of Electroporation System with Measured Bioimpedance: Determining Parameters

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Abstract-The aims of this research is to determine electroporation parameters for a specific biological tissue application. Electroporation is a technique that facilitates the introduction of very small materials into biological cells by applying a high pulsed electric field. The success of electroporation is determined by parameters of the exposure and the properties of tissue. The biological tissue is modeled as a medium with conductivity and permittivity. Both properties are obtained from measurement of the impedance. The whole electroporation system forms an electromagnetic system which appeared as mathematical model of partial differential equation problems. A finite element method (FEM) is used as a tool to solve and simulate the problem. FEM gives graphical presentations showing the potential dan electric field distribution. A map of electroporation that based on electric field exposure is then analyzed to obtain an electroporation area. The results are electroporation parameters in term of electrodes potential different and distance, duration, number, and interval of the pulses. It can be done by choosing the respective parameters that produce certain electroporation area. Some sets of parameters that produce a wide range of electroporation area are also presented.

Keyword: bio-impedance, pulsed electric field, electropora-tion parameters, finite element method, clustering.

I. Introduction

Electroporation is a technique that facilitates the introduction of very small materials into biological cells by applying a high pulsed electric field. The success of electroporation is determined by procedure of the application, parameters of the exposure and the properties of tissue. The biological tissue is modeled as a medium with conductivity and permittivity. Both properties are measured by an LCR meter. The medium is modeled as a system of conductive film to get a mathematical model in the form of partial different equation problems. A Finite Element Method (FEM) is used as a tool to solve the problem.

A recent use of pulsed electric field in medical application is electroporation. According to [5], electroporation occurs when induced electrical energy into cells increase the permeability of cell membrane and leads to form membrane pores. Those pores are then make organic molecule, gen, antibody peptide, or DNA enter into cells. Electroporation can also be used for combining some cells. In reversible electroporation (RE), cell membrane pores will immediately close when electric field disappeared. If electrical energy in the pulse is too big or too long, the cell will damage due to broken cell membrane. It is called irreversible electroporation (IRE). The schematic mechanism of electroporation is shown in fig. 1

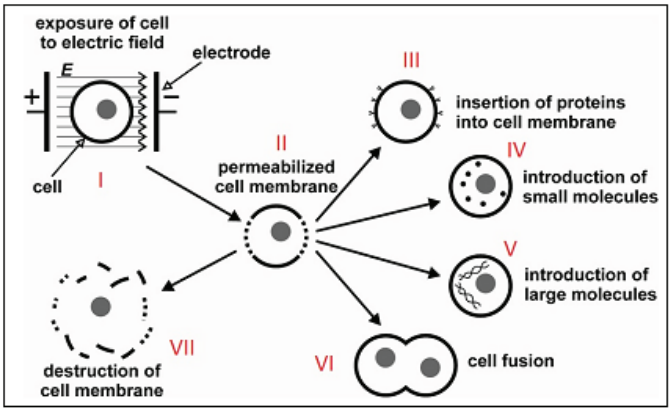

Fig. 1. cell electroporation mechanism [5]

In vivo electroporation is interesting for research because it is the most valuable bur risky procedure. Until now the existing electroporation is based on practical experience from direct experimentations. The problem is how to know that electroporation success as we want, considering minimizing side effect, therefore it is necessary to conduct research for electroporation in detail through numerical calculations to determine the parameters of electroporation.

The research give a simulation of pulsed electric field distribution at tissue in order to know the part of tissue that the cells is electro orated. Furthermore it will also determine the electroporation parameters that give specific electroporated area. The results are expected as design guide of safe but successful electroporation system.

II. MATERIALS AND METHODS

A. Tissue electrical properties

Three important electrical parameters in the interaction between electric field and electrical material are permittivity, conductivity, and permeability. Permittivity, ε, in Farad per meter (F/m) shows the number of polarization and partial rectifying of permanent electric dipole to electric field. Conductivity, σ, in Siemens per meter (S/m) shows number of conduction current density that occurs due to some amount of electric field. The magnetic dipole rectifying is measured by permeability, μ, in Henry per meter (H/m). The Tissue model is electrical impedance model as shown in fig. 2 which is a series equivalent circuit resistance (R_F) and capacitance (C_F).

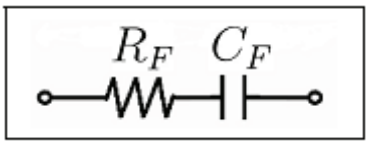

Fig. 2. simple biological tissue impedance model of series resistance and capacitance circuit [1]

For FEM modeling, the biological tissue electrical model is represented by conductivity and permittivity that independent from electrodes distance. Resistance (R_F) and capacitance (C_F) both in series or parallel circuit are real and imaginer component of the impedance. If impedance is $Z = R_F + j/(-\omega C_F)$ with R_F and $1/\omega C_F$ are real and imaginer part respectively. The relation between conductivity (σ) and relative permittivity (ε_r) with impedance represented as (1) [2]:

$$Z = R_F + \frac{j}{-\omega C_F} = \frac{1}{\sigma} + \frac{j}{-\omega \varepsilon_0 \varepsilon_r} \quad (1)$$

with ω is radial frequency and ε_0 is free space permittivity i.e. 8.86×10^{-14} F/cm. Conductivity (σ) and absolute permittivity $\varepsilon = \varepsilon_0 \varepsilon_r$ can be calculated by (2).

$$\sigma = \frac{1}{R_F} \qquad \varepsilon = C_F \quad (2)$$

B. Impedance Measurement

The biological tissues which mostly used as electroporation object especially in drug delivery and vaccine DNA is muscle tissue [9]. The material for the experimentation is a lamb and cow muscle tissue as shown in fig. 3. The tissue sample dimension is with length of 20 mm, width of 20 mm, and height of 2 mm. The volume of tissue would 800 mm^3. The acrylic plate as tissue holder have dimension of 60 mm x 60 mm. The electrode is stainless steel needle with diameter of 0.45 mm. The number of sample is 66, with 30 samples are cow muscle and 36 samples are lamb muscle. The experimentation is conducted about 1-4 hours after the muscle tissue cut from the animal.

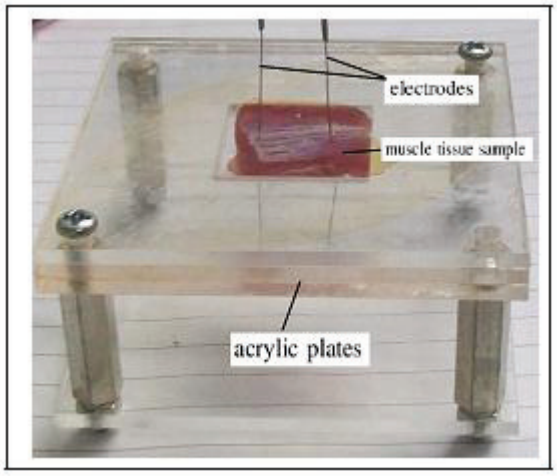

Fig. 3. Sample of muscle tissue in acrylic plates with 2 electrodes

Two electrode are connected to LCR meter made by Tinsley type 6401 to measure the impedance [7]. The impedance consist of resistance and reactance and we expect that the reactance would be capacitance due to dielectric characteristic of the biological tissue. The measurement is about 15 second after LCR meter power up. The resistance and capacitance are then converted into conductivity and permittivity. Two electrode are connected to LCR meter made by Tinsley type 6401 to measure the impedance [7]. The impedance consist of resistance and reactance and we expect that the reactance would be capacitance due to dielectric characteristic of the biological tissue. The measurement is about 15 second after LCR meter power up. The resistance and capacitance are then converted into conductivity and permittivity.

C. Modeling and Simulation using Finite Element Method

The conductivity (σ), permittivity (ε), geometry specification, boundary condition of the material and electrode and voltage source as input for finite element method modeling as used in [8]. Tissue model that to be used for FEM is 2 dimensional square box. The electric filed generated from 2 circle electrodes with space of 10 mm. One electrode is positive voltage and the other has zero voltage. The boundary condition of electrodes are set as Dirichlet and Newman type for the muscle tissue. The main output is potential distribution that can easily obtain potential distribution of electric field distribution. The formulation of electric field can be derived from equations of Maxwell equations of electromagnetic (3)

$$\nabla \times \mathbf{E} = -\mathbf{j}\omega\mathbf{B} \quad (3)$$

and (4)

$$\nabla \times \mathbf{H} = \mathbf{J} + \mathbf{j}\omega\varepsilon\mathbf{E} \quad (4)$$

with E is electric filed intensity, B is magnetic flux density, H is magnetic field intensity, J current density, and " is material permittivity. magnetic flux density can be represented as (5):

$$\mathbf{B} = \nabla \times \mathbf{A} \quad (5)$$

with A is magnetic vector potential

By using equation (5) and (3), electric field E can be expressed as (6)

$$\mathbf{E} = -\mathbf{j}\omega\mathbf{A} - \nabla\phi \tag{6}$$

with - is scalar potential

By using divergence of (4) and Ohm Law, J = σE, and (6) obtained continuity equation as (7):

$$-\nabla \cdot [(\sigma + j\omega\varepsilon)(j\omega\mathbf{A} + \nabla\phi))] = \mathbf{0} \tag{7}$$

With boundary condition of system is (8)

$$n \cdot (\mathbf{J} + \mathbf{j}\omega\varepsilon\mathbf{E}) = \mathbf{0} \tag{8}$$

With n is normal vector unit. In electroporation case, generally, it is assume that magnetic field is very small and can be neglected, therefore A = 0, and (7) become (9). This equation is a form of partial differential equation of electrostatic which represent the characteristic of biological tissue under electrical potential.

$$-\nabla \cdot [(\sigma + j\omega\varepsilon)(\nabla\phi)] = 0 \tag{9}$$

Equation (8) and (9), which are a partial differential equations are readily to be solved by using Finite Element Method (FEM) as shown in [4]. Two dimensional FEM will be used to solve the problem by minimized the objective function (10)

$$F(\phi) = \frac{1}{2}\iint_S \left[\left(\frac{\partial\phi}{\partial x}\right)^2 + \left(\frac{\partial\phi}{\partial y}\right)^2\right] ds \tag{10}$$

With S is area of intersection between integration and wave-form. The main principle of fem is that it divides problem into finite and large amount elements [6]. The elements can be one, two, or three dimension that called mesh. If the area of intersection assumed as small triangle elements then:

$$F(\phi) = \sum_{e=1}^{N_e} \iint_{A_e} \frac{1}{2}\left[\left(\frac{\partial\phi_e}{\partial x}\right)^2 + \left(\frac{\partial\phi_e}{\partial y}\right)^2\right] ds \tag{11}$$

with e is triangle number, N_e is amount of element, and A_e is element area e at the integrated function .

At the point that forms triangle it can be calculated the potential and electric field, with potential boundary condition at positive electrode is of ϕ and at negative electrode is zero.

According to criteria of electroporation as shown in fig. 4 the reversible electroporation (RE) has minimum of 158.49 V/mm and maximum filed intensity of 562.34 V/mm with duration between 0.015849 ms and 2 ms.

D. Electroporation area and parameters

By using the electric field distribution and the criteria in fig. 4, it can be determine in which electric field occur RE and the electroporated area can be obtained by calculation the area which bound by the contour line. Before doing that it is needed that the domain of electroporation is Cartesian therefore triangular mesh should be converted including the value of the potential. The counter line is at the point of the maximum and minimum of the criteria. If volume is needed it is simply by integrating the surface to the thick of tissue. Calculation of electric field intensity for electroporation in a tissue can be used as indication for successfully and safe electroporation.

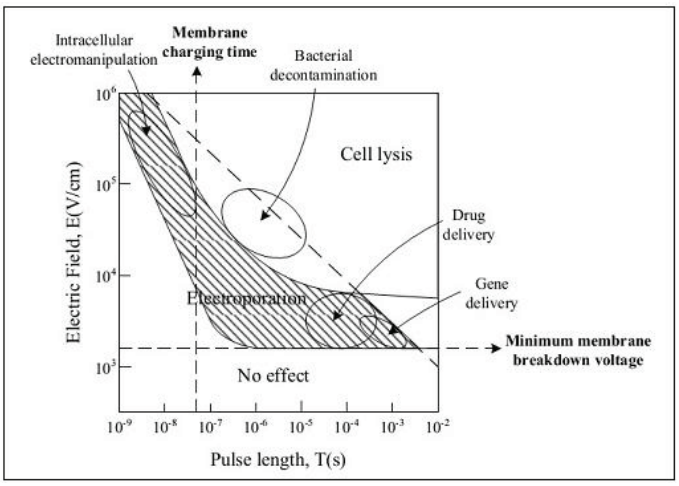

Fig. 4. Parameter range for bioelectric applications (Electric field E Pulse length T) [9]

A map of electroporation that based on electric field exposure is then analyzed to obtain an electroporation area [3]. The results are electroporation parameters in term of electrodes potential different and distance, duration, number, and interval of the pulses. It can be done by choosing the respective parameters that produce certain electroporation area. This parameter should consider the ease of practical design e.g. the very short duration is hard to design in hardware than the long one, the higher voltage need more expensive device, etc. It would be interesting that the result in form of sets of parameters that produce a wide range of electroporation area and easily implemented.

III. Results and Discussions

A. Electrical properties of Muscle tissue

The measurement result is obtained from LCR meter. The results can be summaries as follows:

- there is relatively no different between impedance of cow and lamb muscle tissue, with statistical t-distribution test (t-test) is 0.7.
- The variation of tissue weight and volume do not affect the measured impedance with statistical χ^2 test of $3.5x10^8$
- The average resistance, R is 4369.75 Ohm
- The average capacitance, C is 147.01 nF

The average conductance is therefore σ = 1/R/10 = 0.00022885 S/mm and the average permittivity is ε = C/10 = 1:4701x10^8 F/mm = 14.701 nF/mm

The average conductivity and permittivity σ, ε, with angular frequency of 1000 Hz (frequency of LCR meter), electrodes space of 1 cm (10 mm), combined with other parameters are then simulated using FEM by using equation (8) and (9). The other parameters are varied as follows:

- 30 Voltage variations of 24V-100kV
- 19 duration variations of 0.0000016-0.03s
- 8 variation of pulses number of 1-32
- 14 variations of pulse intervals of 0.000001-1s

From the simulations it is selected by considering the ease of design and implementation and maximum range of application. In the hardware design, to select parameters, it uses selector switch, 6 steps, rotary mechanism for practical use rather than digital set. So for each parameter only 6 choice possibilities and therefore needs careful design to get optimal choices. For 4 parameters i.e. pulse voltage, duration, number, and interval, there is 1296 choice possibility. This choices should have maximum range of electroporation.

B. Electric field distributions map and area of the electroporations

The result of simulation is electric filed distribution and the contours correspond to the boundary of reversible electroporation criteria. The typical plot electric filed distribution is shown in fig. 5. The figure shows electric field distribution in the muscle tissue. It shows 20 % of the square shape tissue is reversible electroporated and the rest are not electroporated. Two white circles show the electrodes with 10 mm space, that has positive voltage at one and zero at other. Electric field distribution and contour express locations that have same amount electric field. Color gradation shows electric field intensity to zero potential reference. Red color show high intensity and blue the low intensity. This simulation is obtained with parameter of voltage of 500V, pulse duration of 0.00005 s, 2 pulses with interval of 0.0002 s. Other experiment with other parameters combination are also experimented.

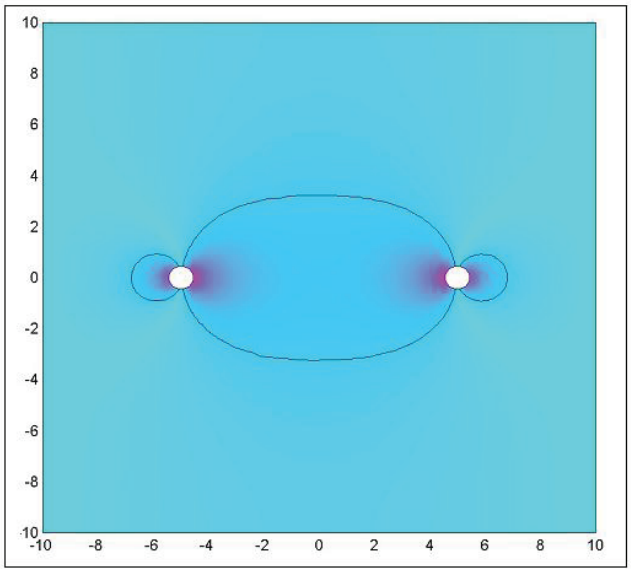

Fig. 5. Map of reversible electroporation with 20 % area

Ideally the pulse generator should capable to generate pulses for all range of electroporation level. But the cost of the hardware will expensive and not practical to the operator. Therefore some simple specification should be established. The specification are shown in table I. It shows the classification of electroporation area. It give 7 classifications of electroporation are: 1 group of not electroporated (NE), 5 groups are reversible electroporation (RE) and 1 group of irreversible electroporation (IRE). This table to make ease of design since in practical very detail range is not the concern. In other words the design of electroporation should satisfy those 7 groups of electroporation.

TABLE I.
SPECIFICATION OF MINIMAL ELECTROPORATION LEVEL

Group	Elektrp. Treatment	Area (% total)		
		NE	RE	IRE
1	0	100	0	0
2	20	80	20	0
3	40	60	40	0
4	60	40	60	0
5	80	20	80	0
6	Max RE	15	85	0
7	Max IRE	0	10	90

It can noted that with the designed system the maximum RE without irreversible condition is around 85 % of area. For maximum reversible electroporation the result is shown in fig.6.

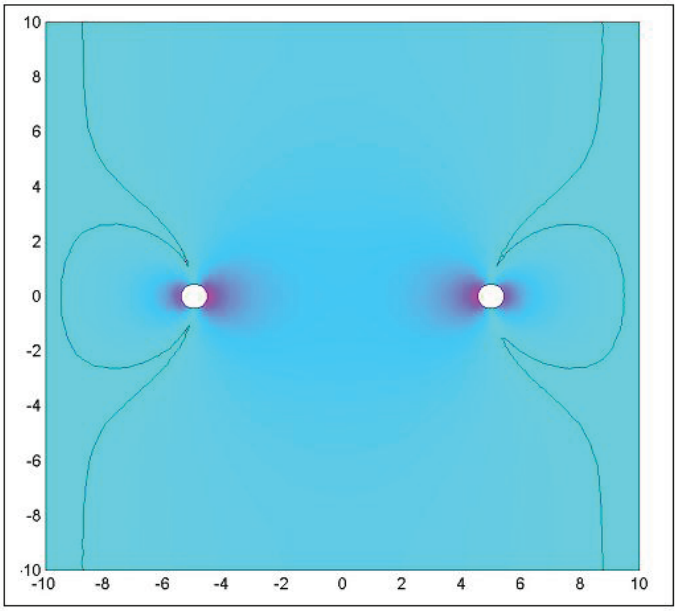

Fig. 6. Map of maximum reversible electroporation area

Fig. 7 shows a map of maximum irreversible electroporation area which mostly IRE. With designed electroporator the maximum irreversible is achieved at 90% area and the rest is RE. The most electroporated was area surrounded electrodes that perpendicular with other electrode.

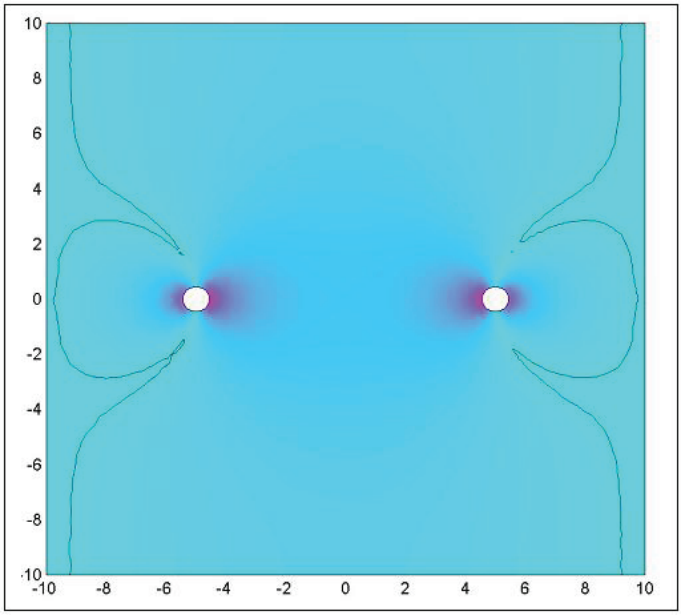

Fig. 7. Map of maximum irreversible electroporation area

C. Electroporation parameters

Some selected simulation results are presented in graphic. It should be selected the parameter combinations that satisfy the specifications.

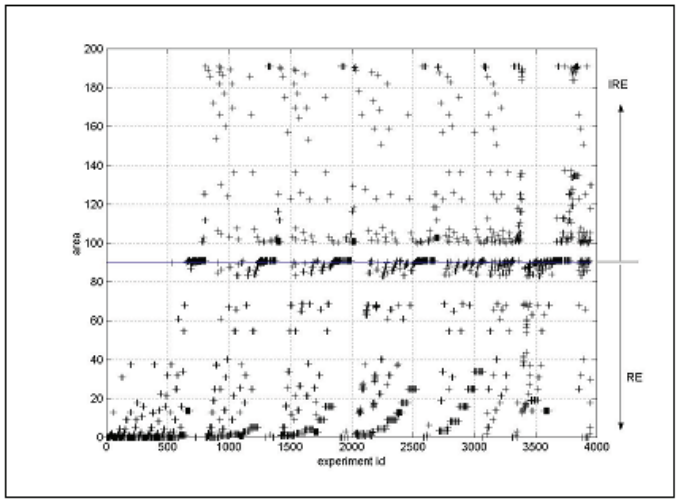

Fig. 8. Electroporation area as a function of experimental sequence

To determine the parameter it is approached by using statistical method i.e. clustering as shown in fig. 9. The frequency of a parameter value appears in all group is calculated and ranked from the highest to lowest score. Then it is simply that the top ranking is the parameters. The result of selected parameters is shown in table II.

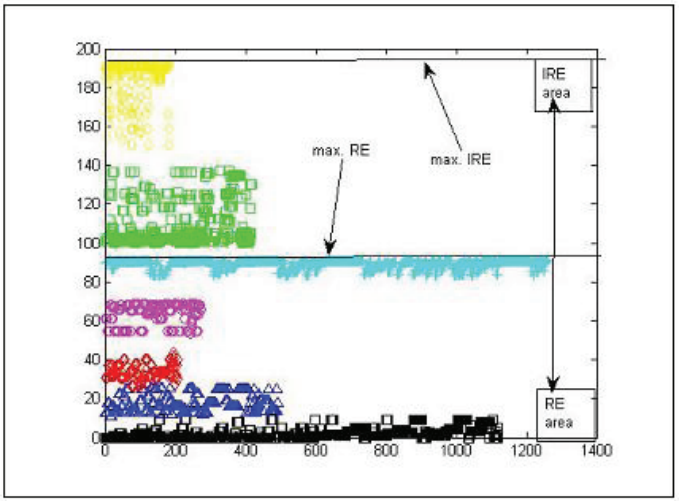

Fig. 9. Clustering of the simulation results

TABLE II.
SELECTED ELECTROPORATION PARAMETERS

Paramt	Unit	Parameter selection					
		1	2	3	4	5	6
Amplitde	Volt	200	300	400	500	600	1200
Duration	ms	0.05	0.06	0.07	0.09	0.15	20
Interval	ms	0.2	2	20	200	500	1000
Number		1	2	3	5	8	16

IV. CONCLUSIONS

Modeling and simulation of electroporation system give description of the potential and electric field distribution at biological tissue due to pulsed electric field. Based on measured electrical properties of a tissue, the level electroporation can be mapped and the electroporated area can be calculated.

With muscle tissue of 20x20x2 mm the maximum reversible electroporation area is 80 % and irreversible electroporation is 85% of total area with designed system. The electroporation parameters in term of electrodes potential, duration, number, and interval of the pulses are then simulated by simulations using FEM to get optimal parameters. Simulations of all possible parameter combinations determines the designed electroporation parameters, by choosing the respective parameters that produce certain electroporation area. The final results are some sets of parameters of 6 voltage, 6 duration, 6 interval, and 6 number of pulse that produce a wide range of electroporation satisfy 7 groups of electroporation levels.

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