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A CRITICAL ANALYSIS OF ELECTROPORATION IN MEDICAL TECHNOLOGY

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ABSTRACT: When a high amplitude nanosecond electric field applied to a biological cell it disrupts the equilibrium of the phospholipid bilayer resulting in the permeability of the cell membrane to increase. This results in movement of extracellular material into the cell which otherwise cannot move inside the cell. This phenomenon is known as electroporation. In the initial years of research people concentrated on to find out the process which leads to the above phenomenon, but till now it is not proved theoretically / mathematically the complete theoretical background about the process. In recent years with the help of new fundamental knowledge and the latest experimental electroporation researchers can shift the technologies based on electroporation for clinical validations. In this paper, we have discussed regarding the theories of electroporation and also regarding the usages of electroporation in medical technologies. We have also discussed the challenges to be faced and the research barriers to make this technology on the market.

INTRODUCTION: Electroporation involves increasing the membrane pliability with the applied shortened duration higher electric field. Increasing cell permeability allows inflow of extracellular material into the cell which otherwise could not enter the cell. Electroporation can be explained theoretically with the help of a transient pore model. It says that aqueous pore is created in the cell membrane when an electric field is applied. It also says that cell membrane is made up of phospholipid bilayer which mainly consists of a hydrophobic head, hydrophilic tail, proteins, and carbohydrates. It states that when no electric field is applied, then there exists an equilibrium between the forces of the lipid heads which can be classified as surface tension and edge line tension.

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When the cell exposed to the field, this dynamic equilibrium of the lipid forces disrupts which leads to cell membrane pore creation. It observed that the pore created due to the application of an external electric field is generally hydrophilic in nature and it is generally formed in a small section of the cell where the field is applied. There are a lot of efforts made to visualize the change in the structure of the membrane, but at present, there is no such literature regarding it [1].

The history of systematic observation of electroporation started in the eighteenth century by Nollet [2] when he observed red spots over the animal skin on the application of the electric field. The initial work on electroporation is evitable from the year 1999 when electroporation was evaluated using molecule interaction studies, and a few years later, experiments were performed, but the hypothesis could not be validated with theoretical explanations. Experiments using nanoseconds pulses conclude that the increase in conductivity can be related directly to pulse duration and amplitude of electric field [3].

As Axel [4] analyzed the conductivity and permeability changes in a spherical cell when an electric pulse is applied. It also includes studies of post-pulse behavior to find the parameters which affect pore stabilization and the relation of long-lived and transient pores. In patent [5] the inventors achieved modality of cell death by using a bipolar pulse of high frequency. More specifically it is claimed that the effect of bipolar electrical pulses of bursts width of microsecond's duration of a single polarity. A study by Lamberti [3] confirms the effect of pulse repetition in electroporation induced by nanoseconds pulses electric field in mammalian cells. In the patent [6] the inventors studied methods for non- thermal irreversible electroporation to cause cell death in treated tumors and ultra-short electric pulses in the temporal and spatial domain are used to achieve the desired modality of cell death. Recently, Miklavcic [3] studied skin electroporation, and the results indicate that high voltage electric field of shorter duration created a pore in the cell membrane. It also creates local transport region in stratum corneum and the layers of the skin. Last few decades shows the usage of electroporation in the field of medicine, food processing, and pharmaceutics. In the year 1982 performed seminal work of delivery of foreign DNA into the cell by using the pulsed electric field.

Later on, more studies using nanosecond pulsed electric field led to the development of more clinical applications such as gene electrotransfer (GET) and electrochemotherapy (ECT). In recent years another application of electroporation in medical application emerged is ablation of the tumor by the use of non-thermal irreversible electroporation (NTRE).

Fundamental Principles of Electroporation: Electroporation is defined as the process of increasing the permeability of the cell membrane by applying short duration high voltage electric field as shown in **Fig 1**. This helps in the introduction of molecules which are unable to enter through the cell membrane. The process of electroporation can be classified into three different phases. The first phase accounts for the formation of a pore. This is basically the response of cell membrane to increase in the potential of the membrane, and it lasts in the range of microseconds.

The second phase of electroporation accounts for the increase in pore size which takes place in milliseconds duration. This completes as longs as the field is applied. The last phase of electroporation accounts the resealing of the pore when the field is withdrawn. This lasts for about several minutes after removal of the pulse [7]. Applications of electroporation spread in the field of medicine, biotechnology, and food processing. Drug delivery, tumor treatment, and electro-chemotherapy are the most prominent applications of electroporation in medical technology.

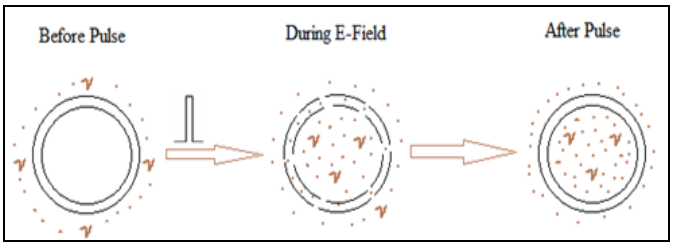

FIG. 1: DESCRIPTION OF THE PROCESS OF ELECTROPORATION

Transmembrane Voltage and Electroporation: Electrically, a biological cell can be modeled as an electrolyte (also known as cytoplasm) which is covered by an insulating layer (also known as plasma membrane). Transmembrane voltage can be generalized as the voltage developed at the membrane of the cell when it is brought to the electric field. The external electric field is applied using electrodes which are placed closer to the cell. This configuration is known as patch clamp configuration, and the induced transmembrane voltage (ITV) in this configuration is reported to be constant over the exposed patch [8]. In a spherical cell, ITV can be represented with the help of Schwan steady state equation [9, 10].

$$TMV\ (V_m) = 3/2\ ER \cos\theta \quad \text{.....1}$$

$$TMV\ (V_m) = \Delta\Psi_m = f_s\ ER \cos\theta \quad \text{.....2}$$

Where, E is an electric field, R is the radius of the cell, θ is angle bounded by electric field and radius of the cell and is the distribution of electric field. The above equations are used to solve the ITV, and if the value of ITV changes from 0.8 V (Resting Potential) to 1.0 V then it can be concluded that the cell is electroporated as shown in the **Fig. 2**.

f_s is a dimensional less factor, and a simplifying assumption obtains the value of it that the

membrane is a pure insulator, σ_m = 0 and the function turns into a constant,

$f_s = 3/2$ 3

Where σ_m is the membrane conductivity of the spherical cell.

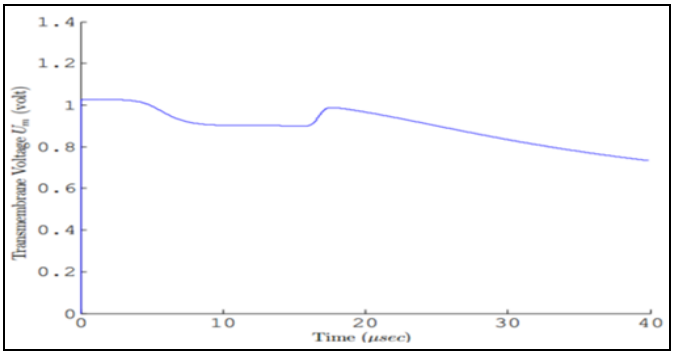

FIG. 2: ITV OF A SPHERICAL CELL

The equation (1) describes the steady-state behavior which comes microseconds after the application of the field. Generally, DC electric field lasts for about hundreds of microseconds thus equation (1) can be used to calculate the steady-state value of ITV. It is also evident that ITV is directly proportional to the applied electric field E, Radius of the circle R and cosine of the angle between the direction of applied electric field and the radius of the cell. The spatial distribution of ITV is such that it has a peak value at the poles and it is equal to zero at the equator [11, 12].

For studying the transient behavior of initial microseconds in addition to electrical conductivity, we have to consider the dielectric permittivity of the membrane, ε_m.

$\Delta \Psi_m = 3/2\ ER \cos\theta\ (1- e^{-t/\tau})$ 4

Where τ is membrane charging constant and its typical value is 0.5 μs, and t is the time duration of an applied electric field.

Numerically ITV can be estimated in the case of unevenly shaped cells as the variation among the potentials on the inner side and the outer side of the membrane.

$\Delta \Psi_m = \Psi_i (t) - \Psi_e (t)$ 5

The numerical method of determination of ITV provides a good approximation, but it does not match completely with the experimental results because of a lot of approximations in the numerical simulation. An alternative approach to numerical simulation approach of calculating ITV is an experimental analysis of ITV using the potentiometric dye [13, 14]. The formation of a pore in the cell is directly correlated with a value of the membrane potential exceeding a certain fixed value. Thus, we can conclude that the membrane transport is directly dependent on the value ITV generated by the external electric field [15]. The correlation and dependency of ITV and membrane transport through an electroporated cell can be demonstrated clearly by monitoring of ITV concerning an electric field applied and later validating it with the experimental result using potentiometric dye.

Pore Formation and Resealing: The formation and development of pores are related to the stress development in the membrane. The cell membrane consists of phospholipid bilayer which consists of a hydrophilic head and hydrophobic tail. When no electric field is applied the hydrophobic tail, and hydrophilic head remains in dynamic equilibrium. This dynamic equilibrium is the result of the repulsive force of lipid heads and surface tension of the pore perimeter as shown in the **Fig. 2**.

When the cell is exposed to an applied electric field, the field disrupts the equilibrium of the steric repulsion forces and thus the surface tension increases leading to an increase in line tension and pore formation. The kinetics of formation of the pore can be characterized by an equation:

$dN / dt = \alpha e^{(ITV/Vep)^2} (1- N/N_0\, e^{-q(ITV/Vep)^2})$.....6

Where α is pore creation coefficient, N_0 is the pore density of non-electroporated membrane, ITV is the induced transmembrane voltage, V_{ep} is the electroporation voltage, and q is the electroporation constant. This equation gives the value of the density of pore being created on the surface of all the membranes. The creation of pores raises conductivity of membrane which is referred to as conductivity due to electroporation (σ_{ep}). The formation of pores takes place within nano-seconds to microseconds time duration after the application of the electric field. However, the resealing of pores takes a longer time as compared to pore formation time after the electric field is removed [16].

Detailed experimentation reveals that the pore formation and resealing process takes place in several steps and each step takes micro-and/or milliseconds time duration [17].

Evolution of Pore Radius: For a cell with k number of pores the amount of transformation of their radii r_j is resolved by the specified ODE as follows:

$$\frac{dr_j}{dt} = U(r_j, V_m, \delta_{eff}) = \frac{D}{kT}\left(\frac{V_m^2 F_{max}}{1+r_h/(r+r_t)} + 4\beta\left(\frac{r^*}{r}\right)^4 \frac{1}{r} - 2\pi\gamma + 2\pi\,\delta_{eff}r\right)$$

J = 1, 2, 3 ……. K 7

Where, r is the radius of the cell, r^* is the minimum radius of the hydrophilic pores, V_m is ITV, F_{max} is maximum electric force, β is maximum steric repulsion energy, γ is stress of layer after the pores, σ_{eff} is constant for the direct stress of the membrane, r_h and r_t are constants for advection velocity and k is Boltzmann constant.

Molecular Transport in Electroporated Cell: Molecular transport in the electroporated cell is calculated using the Fick's law with molecular migration in porous media under an electric field.

The equation of Fick's law is as follows:

$$R_i = \delta C_i / \delta_t + \Delta.\ (-\Delta C_i - z_i.\ u_{m,i}.\ FCi.\ \Delta V) \text{ .....8}$$

$$N_i = -\ Di\ \Delta\ C_i - z_i\ u_{mj}\ FC_i\ \Delta V \text{ .....9}$$

Where V is electric potential, R_i is reaction rate expression; C_i is a concentration of the molecules in the solution, D_i is diffusion coefficient, $u_{m,i}$ is the mobility of the molecules and z_i is the charge number.

Application of Electroporation in Medicine:

Electrochemotherapy (ECT): Electrochemotherapy is a non-thermal treatment for tumors which mainly consists of an application of nano-seconds pulsed electric field combined with anti-cancer molecules [18, 19, 20]

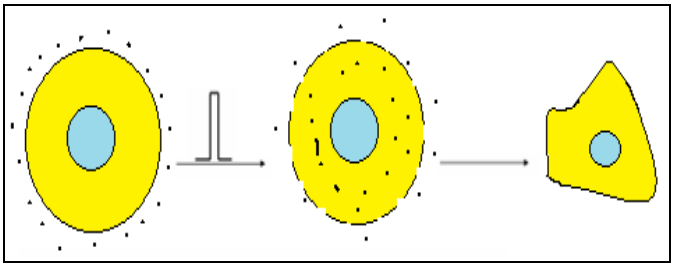

FIG. 3: DESCRIPTION OF ECT

The POC of this procedure is done both in simulation and animal model experiments, and in the later stage of development, it was applied to the human model. A reversible EP is applied to the treatment region which is meant to permeabilize the cell in that region to allow administering the anti-cancer drug directly into the tumor cell or systematically as shown in the **Fig. 3**.

The main advantage of electrochemotherapy is that this treatment technique is very selective in choosing the targeted tumor cell and field is only applied to specific cell followed by administration of the anti-cancer drug. The first fundamental examination of ECT released in 1991. In this paper, the authors studied the feasibility of ECT treatment procedure. They have also studied the safety norms required to perform ECT. Based on the initial clinical studies researchers from United States (Tampa), France (Toulouse) and Slovenia (Ljubljana) started to study and perform further clinical experiments [21, 22]. This experimental study led to the publication of a joint paper in 1998. [23]

The European government started a project named" European Standard Operating Procedures on Electrochemotherapy (ESOPE) to generalize the procedures of clinical use of electrochemotherapy. Standard Operating Procedure (SOP) of the ECT along with the pulse generator cliniporator was published in the year 2006. [24] ECT gained wide acceptance as clinical practice in Europe, and clinical findings were published in 2008 [25] and data from the findings were systematically reviewed and analyzed [26] the result of which was published in 2012. Analysis of the data proved that ECT has a higher effectivity (more than 80%) than bleomycin and cisplatin alone. The total effectivity (including complete and partial responses) of ECT was 84.1%. In current state, ECT is being used to treat around 130 cancer patients in Europe [27]. It is generally being used to local tumors. In addition to, it is also being used for the treatment of metastases in veterinary oncology [28].

Non-Thermal Tissue Ablation (NTRE): In NTRE high intensity pulsed electric field is being applied to the target cells which cause irreversible damage to cell membrane. This treatment procedure is location specific; thus, it does not cause any harm to the surrounding healthy tissue and the blood

vessels [29, 30, 31]. The major advantage of this technique is the preservation of the healthy tissue and thus reducing the chance of scarring (as reported [32, 33]). The first preliminary work on NTRE was released in 2005. Here, the author used the mathematical model to predict NTRE. The results of the simulation suggested that NTIRE develops a significant amount of tissue ablation non-thermally. The result of these mathematical models was later proven by experimental studies of laboratory cell cultures and intestinal models. The results of the simulation were recently verified by first human model studies [32, 33]. The perfect cause of cell death by NTRE is still unknown to the researchers and the reports on the pathway of cell death caused by NTRE are contradictory to some extent. A very limited attention has been provided to the *in-vivo* study of inflammatory responses to NTRE. Al-Sakere *et al*., [34] reported in his paper that NTRE does not induce infiltration to immune cells into the treated tissue thus it can be concluded that immune response is not essential for the treatment of NTRE.

Gene Electro Transfer (GET): Gene electrotransfer is mainly used for DNA vaccinations or for the treatment of different cancers where the therapy is directly targeted to the tumor cell to escalate the response of cancer cells. Generally, DNA vaccinations using electroporation is done intramuscularly using needle electrodes. A very few researchers have focussed on FEM simulation of GET mostly the people have performed preclinical and clinical studies to gain more insight into the technology. Arena *et al*., [35] discussed simulation of electroporation in tissues, and he also discussed the importance of a model that is used in the model. He also discussed the results of the simulation with experimental clinical studies. Fischer *et al*., [36] discussed the simulation and experimentation of electroporation-based delivery of DNA vaccines.

Here, the authors compared the result of two Fem simulations: Simulation has parallel plate electrode and other with invasive needle electrodes. The results of the simulations showed that the parallel plate electrode if oriented properly, can concentrate the electric field effectively within the adipose tissues. The simulation result was later verified by the experimental observations.

Potential and Challenges: Electroporation results in an increase of cell membrane permeability using a chemical free and non-thermal path. Thus, electroporation can be used to (a) Programming of cell functions with new external molecules affecting cellular pathway (b) Causing programmable cell death (c) Inserting new materials in the cell. The review given the paper suggests that the experimental results of NTRE, GET and ECT are encouraging enough to get it through the human trails, but still the major challenge that the researchers are facing is the lack of a clear concept of the behavior of cells and tissues under an applied electric field.

CONCLUSION: Electroporation is an integrative technology with numerous numbers of medical applications. To successfully apply the technologies of electroporation it requires an impactful collaboration of persons from different domains such as medical sciences, computer science, and electronics engineers. Currently, available knowledge of electroporation propounds that electroporation has numerous potential and it can solve problems of medical sciences globally which includes vaccination, cancer treatment, and treatment of other infectious diseases. Although, a lot of researchers are working in the field of electroporation, complete knowledge about the fundamental mechanism is still to be developed. In the coming years, there will be a huge increase in the number of medical treatments based on electroporation.

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CONFLICT OF INTEREST: Nil

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