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Review Article

Electroporation: An Emerging Technique For Drug Delivery

Paria U*, Bhangi A.
NIMS Institute of Pharmacy, NIMS University, Shobha Nagar, Jaipur-303121, Rajasthan, India
* ***Corresponding Author*** Email: utpalparia@gmail.com Phone: +918890155990
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ABSTRACT

Electroporation is an emerging field in delivery of drugs into the body by any medium or vector. Now a day, it is widely used in many routes for the treatment of diseases. Electroporation is a process in which an electric field is applied to the living cell which results in formation of pores in cell. In this method, viruses or plasmids enter into the cell in the form of vector. For this method instruments like Eppendorf Eporator are used to provide a fast, simple and safe path to administer bacteria, yeast or any other micro-organism as a vector inside the body with DNA/RNA. This technique offers a large number of applications in various fields such as DNA transformation, induced cell diffusion, in transdermal drug delivery, chemotherapy, gene therapy etc. This review deals with the applications, recent developments future scenario of this process. Electroporation knife is the latest invention used to kill the cancer tumors. Feasibility of transdermal electroporation for insulin loaded nanocarriers is another achievement. This approach may also use in nuclear targeting. Countless researches still going on to produce an efficient, safe and simple process for treatment of diseases and provide a better future to the human beings.

Keywords: Electroporation, Iontophoresis, Microneedle, Penetrationenhancer, Sonophoresis, Transdermal.

INTRODUCTION

Electroporation is the process of biotechnology to pass the electric current through the living surface from example, a cell or a molecule. Through this way, pores appear in the surface of the living structure and biological material can pass through it easily. This method is usually used to enter the viruses or plasmids in the form of vectors or plasmids into the cell through pores in the cell membrane.

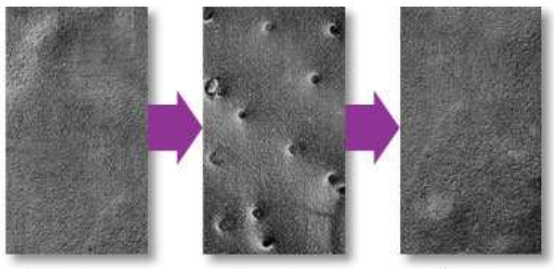
Electroporation, or electropermeabilization, is a significant increase in the electrical conductivity and permeability of the cell plasma membrane caused by an externally applied electrical field. It is usually used in molecular biology as a way of introducing some substance into a cell, such as loading it with a molecular probe, a drug that can change the cell's function, or a piece of coding DNA.

It is a mechanical method used to introduce polar molecule into a host cell through the cell membrane. In this procedure, a large electric pulse temporally disturbs the phospholipid bilayer, allowing molecules like DNA to pass into the cell. It is purely method, which aids a variety of agents from drugs to genes to be an effective means of delivery therapeutic agents to combat disease.

Electroporation has proven useful both in vitro, in vivo and in patients, where drug delivery to malignant tumors has been performed. Whereas initial electroporation procedures caused considerable cell damage, developments over the past decades have led to sophistication of equipment and optimization of protocols. The electroporation procedures used in many laboratories could be optimized with limited effort.

WHAT IS ELECTROPORATION?

The technique of Electroporation is mostly used in the field of molecular biology. When this technique is applied on any cell by using electrical current from an external source, the cell membrane becomes more permeable allowing foreign objects enter into it. When the scientists have to insert a molecular probe, small piece of DNA or any drug which can change the function of the cell, they use this technique **(Figure 1).**



(a) **(b)** **(c)**

Figure 1 The phenomenon of electroporation a) cell membrane, b) cell membrane during pulsing (Controlled, millisecond electrical pulses induce temporary pores in the cell membrane) and c) cell membrane after pulsing (Cell membrane reseals and left unharmed).

It is not easy to make pores in the cell membrane until it is exposed to proper electric field which can allow the plasma membrane to cross its dielectric strength. If the whole experiment is well controlled then after sometime, the pores of the plasma membrane can reseal, but during that time the molecules or foreign objects can enter the cell's cytoplasm. It is harmful for the cell if it is exposed to the electric current for long period of time. It results in the cell death or apoptosis. Process of Electroporation is used mostly for the transformation of bacteria, plant protoplasts and yeast. Bacterial cell wall is made up of peptidoglycan and its derivatives. It has pores in its cell wall naturally, so when the plasmids have to enter into the bacterial cell, a small amount of electric current is used for this purpose just to let the plasmid enter into the bacterial cell. The whole process should be well controlled so that it can be observed

that the bacterial cells can divide into new daughter cells containing the plasmids. This process is more effective than the chemical Electroporation. This process can also be used for the tissue culture cells to enter the foreign genes into the mammalian cells mainly.

Principle: Electroporation (EP) is the physical process of inducing transient permeability of biological membranes by short pulses of electric fields.1 The most important parameters for effective EP are the electrical field strength [V/cm] and the length of time the field is applied (pulse length) shows the basic relationship between these parameters.

When the applied field strength is high enough and of sufficient duration, aqueous pores will be formed through which molecules that are normally unable to penetrate the membrane can be exchanged between the inside and outside of the cell. These conditions are shown shaded in **(Figure 2).** Too high field strength and pulse lengths lead to an unstable situation where the cell dies and disintegrates (lyses). If the field strength is too low, the breakdown transmembrane potential is not achieved. Similarly, if the pulse length is too short, the membrane cannot be charged enough to reach the electroporation membrane potential. Field strength and pulse length can compensate each other to a certain extent. However, optimal parameters differ for different molecules and cells. For the delivery of low molecular weight drugs into mammalian cells, fields of 1000 V/cm and pulse lengths of 100 ms are usually effective, whereas for the delivery of genes, lower fields and longer pulses yield better results. The classical theoretical model of membrane electroporation most likely does not apply to the delivery of DNA.

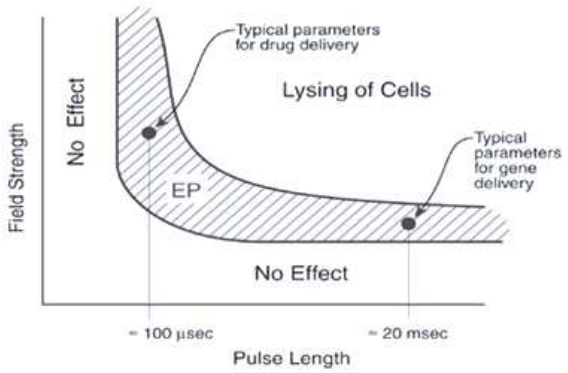


Figure 2 Relationship between field strength and pulse strength as it applies to successful EP of cell

The electric field in a tissue is generated by a potential difference (voltage) applied between electrodes surrounding or adjacent to the target tissue. A wide variety of electrode configurations have been developed and medical grade electrodes are currently being used in medical trials.

BACKGROUND

This image shows the chemical components of the plasma membrane. The polar head groups face outward while the hydrophobic tail groups face inward and interact with one another to hold the membrane together. Polar molecules cannot pass through this membrane without external aid.

Many research techniques in molecular biology require a foreign gene or protein material to be inserted into a host cell. Since the phospholipid bilayer of the plasma membrane has a hydrophobic exterior and a hydrophobic interior **(Figure 3),** any polar molecules, including DNA and protein, are unable to freely pass through the membrane.

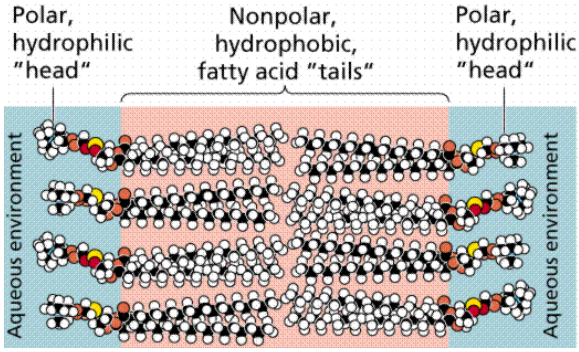


Figure 3 Diagram of the Phospholipid bilayer.

INSTRUMENTATION

The Eppendorf Eporator offers a fast, simple and safe way to transform bacteria, yeast and other microorganisms with DNA/RNA. Results are highly reproducible and compared with chemical methods, electroporation yields to significantly higher transformation efficiency.

Exposing bacteria or yeast strains to short, high voltage electrical pulses enables macromolecules, such as plasmid DNA, to diffuse into the cell through temporary pores in the cell membrane. Designed to deliver ideal conditions for electroporation of bacteria and yeast, the Eppendorf Eporator has been shown to give transformation efficiencies ten times higher than with chemical transformation (heat shock method).

The transformation of bacteria in order to amplify recombinant DNA is often carried out using chemical transformation. The instrument is extremely user friendly. Two new program buttons allow storage and recall of most although reliable and cost-effective, this method is very time-consuming and the transformation efficiency can be too low. The new Eppendorf Eporator not only saves valuable time and delivers ten times higher transformation rates, crucially commonly used parameters and simple one-button operation ensure intuitive use for faster sample handling.

Eppendorf Eporator**(Figure 4)** has a compact, space-saving design for easy storage and transport and comes with a USB port facilitating export of data for analysis and GLP-compliant documentation.

Figure 4 Eppendorf Eporator

This new electroporator incorporates special safety features which maximize user protection. Safe electronics and an integrated electroporation chamber eliminate voltage leaks. Eppendorf electroporation cuvettes are available in three sizes and with different gap widths.

WORKING PROCEDURE

The host cell and the molecules to be inserted into these cells are suspended in solution. The electroporation apparatus is typically commercially produced**.**

When the first switch is closed, the capacitor charges up and stores a high voltage. When the second switch is closed, this voltage discharges through the liquid of the cell suspension [1]. Typically, 10,000-100,000 V/cm (varying with cell size) in a pulse lasting a few microseconds to a millisecond is necessary for electroporation. This electric pulse disturbs the phospholipids bilayer of the membrane and causes the formation of temporary aqueous pores.

The electric potential across the membrane of the cell simultaneously rises by about 0.5-1.0 V so that charged molecules (such as DNA) are driven across the membrane through the pores in a manner similar to electrophoresis.

The actual entry of DNA into the cell cannot be observed with a microscope, but this artist's rendering shows the basic concept of the formation of pores in the membrane through which DNA can pass.

As charged ions and molecules flow through the pores, the cell membrane discharges and the pores quickly close, and the phospholipids bilayer reassembles [2]. The intended molecules should now be inside the cell for further use or study.

Advantages of Electroporation

1) Versatility: Electroporation is effective with nearly all cell and species types [3].

2) Efficiency: A large majority of cells take in the target DNA or molecule. In a study on electrotransformation of E. coli, for example, 80% of the cells received the foreign DNA [4].

3) Small Scale: The amount of DNA required is smaller than for other methods [5].

4) In vivo: The procedure may be performed with intact tissue [6]. A paper published in Developmental Biology showed the successful transfer of a DNA construct with a fluorescent reporter gene into intact mouse brain tissue.

Disadvantages of Electroporation

1) Cell Damage: If the pulses are of the wrong length or intensity, some pores may become too large or fail to close after membrane discharge causing cell damage or rupture [7].

2) Nonspecific Transport: The transport of material into and out of the cell during the time of electropermeability is relatively nonspecific. This may result in an ion imbalance that could later lead to improper cell function and cell death [8].

APPLICATIONS OF ELECTROPORATION

1) DNA Transfixion or Transformation:

This is likely the most widespread use of electroporation. Specific genes can be cloned into a plasmid and then this plasmid introduced into host cells (bacterial or otherwise) in order to investigate gene and protein structure and function. [9]

Bacterial cells already containing a plasmid may be incubated with another strain that does not contain plasmids but that has some other desirable feature. The voltage of electroporation will create pores, allowing some plasmids to exit one cell and enter another. The desired cells may then be selected by antibiotic resistance or another similar method [10]. This type of transfer may also be performed between species. Thus, large numbers of plasmids may be grown in rapidly multiplying bacterial colonies and then transferred to yeast cells by electroporation for study [11].

3) Induced Cell Fusion: The disruption of the membrane that occurs with the quick pulse of electricity in the electroporation procedure has also been shown to induce fusion of cells.

4) Trans-dermal Drug Delivery: Just as electroporation causes temporary pores to form in plasma membranes, studies suggest that similar pores form in lipid bilayers of the stratum corneum- the outermost dead layer of skin. These pores could allow drugs to pass through to the skin to a target tissue. This method of drug delivery would be more pleasant than injection for the patient (not requiring a needle) and could avoid the problems of improper absorption or degradation of oral medication in the digestive system [12].

5) Cancer Tumor Electro chemotherapy: Scientists are investigating the potential of electroporation to increase the effectiveness of chemotherapy. As in electroporation for DNA transfection, the applied electrical pulse would disrupt the membrane of the tumor cell and increase the amount of drug delivered to the site. Some studies have suggested that increased tumor reduction is seen when this method is applied to cancerous cells in animal model systems [13].

6) Gene Therapy: Much like drug delivery, electroporation techniques can allow vectors containing important genes to be transported across the skin and into the target tissue. Once incorporated into the cells of the body, the protein produced from this gene could replace a defective one and thus treat a genetic disorder **(Figure 5)** [14].

7) Vascular Therapy: Electroporation offers a novel approach to overcome the specific difficulties of drug and gene delivery encountered during vascular therapy [15]. This is because EP permits the intracellular deposit of relatively high local concentrations of drugs or genes either into vascular tissue or at sites in need of revascularization due to peripheral or coronary arterial disease. Using a specialized porous balloon EP catheter (Figure 6).

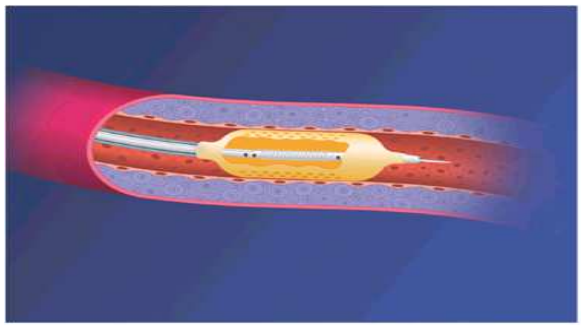


Figure 6 Schematic presentation of the porous balloon EP catheter used to deliver drugs or DNA in to the inner walls of blood vessels

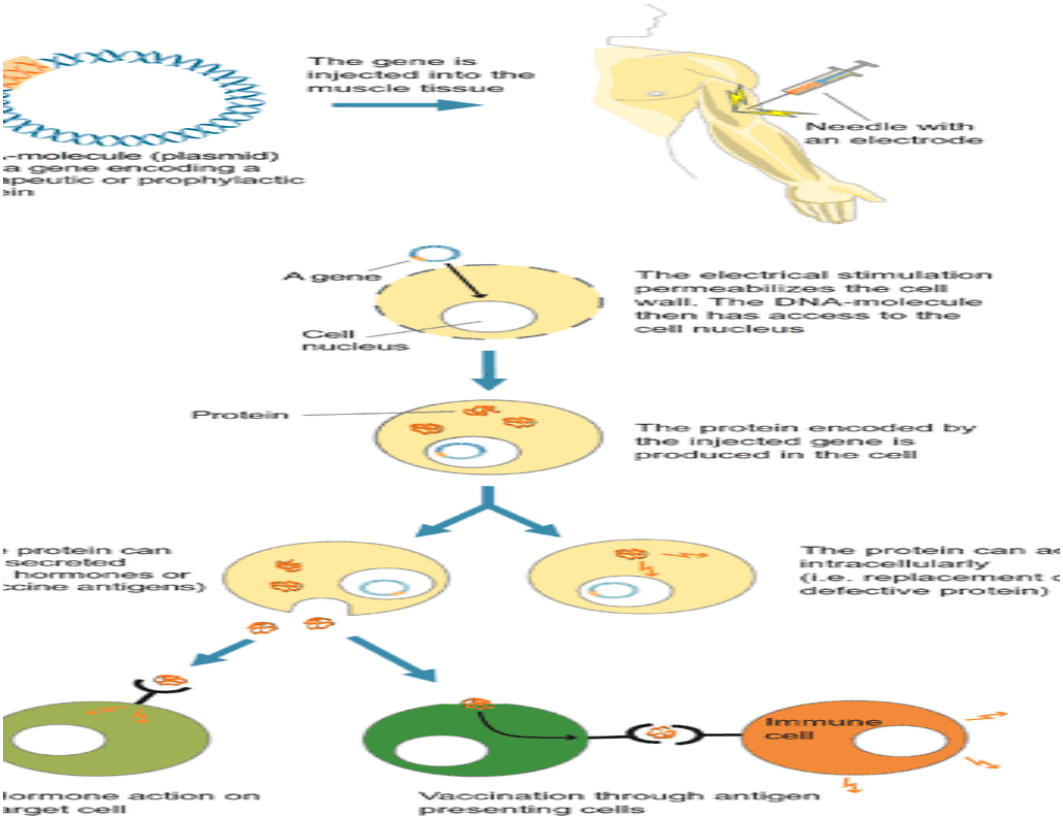


Figure 5 Method of gene therapy using electroporation.

Recent Advancements In Electroporation

1) Electroporation Knife for Cancer:

A new electrical approach to cutting out cancerous tumors **(Figure 7).** This electroporation device can kill cancerous tumor cells with remarkable specificity while inflicting little or no damage on surrounding structures and causing no pain for the patient. Stephen Kee, a radiologist at the University of California at Los Angeles Medical Center, who has been testing the device with guidance and funding from AngioDynamics. The NanoKnife delivers quick bursts of energy through a set of electrodes inserted into and around the tumor. These pulses can last up to 100 microseconds and create an electrical field of up to 3000 volts per centimeter, because of which an electric field will form pores in fatty cell membrane which allow ions to pass through. Nano Knife has already been approved in the United States for use in the ablation of soft tissue, and AngioDynamics has installed prototypes in 17 medical centers around the world, 5 of which are actively using it. The device hasbeen tested so far on 37 patients.

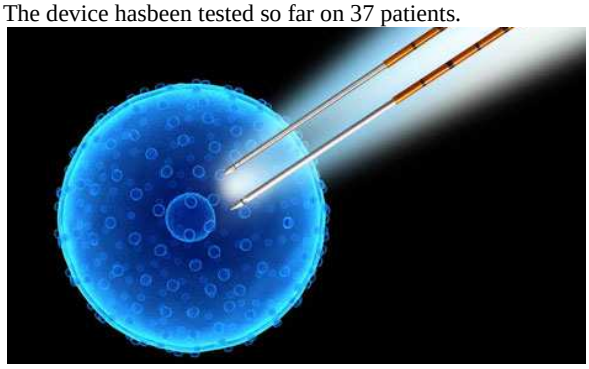


Figure 7 Electroporation knife

2) Electroporation in Transdermal Drug Delivery System:

Electroporation (Figure 8) involves the use of large transmembrane voltages caused by electric pulses (10 µs–100 ms) which create reversible pores in the membrane. It has been recently explored as a potential transdermal drug delivery technique to compromise the obstacle function of the stratum corneum. Recently Rastogiet alinvestigated feasibility of transdermal electroporation for insulin-loaded nanocarriers. It was observed that electroporation of nanoparticles resulted in enhancement in insulin deposition in rat skin. Finally the conclusion was that electroporation of polymeric nanosystems can be successfully used as transdermal drug delivery system for the delivery of insulin loaded nanocarriers.

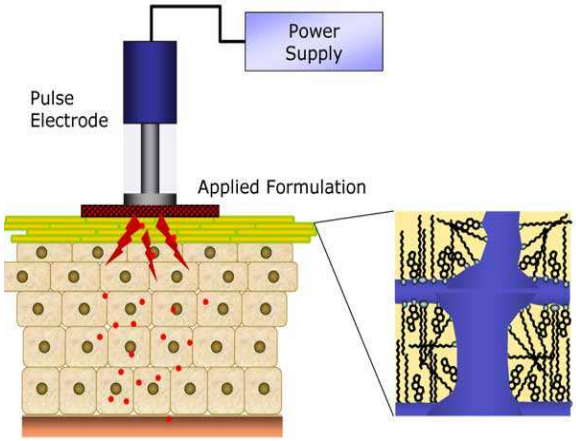


Figure 8 Electroporation in transdermal drug delivery system.

3) Nuclear Targeting: [16]

Most recently, nuclear-targeting peptide scaffolds have been conjugated and synthesized for lipid-based transfection of non-dividing mammalian cells; greater than 80% delivery efficiency and a 63-fold increase in reporter gene expression was achieved 79. Since the final destiny of transfection is the nucleus, it is clear that progress in understanding and exploiting nuclear targeting should greatly increase the efficiency of DNA delivery.

FUTURE PROSPECTIVES

Advances and researches in the field of electroporation are still going on to get more efficient and sophisticated results. A modified type of electroporation, using a low fixed voltage, made it possible to enhance cell viability. In mouse embryos, it would be useful to be able to introduce DNA into cultured mouse embryos in a similar manner by electroporation. In diverse tissues of mice, it would be helpful to be able to vary this method within the CNS and extend it to other tissues. As the electroporation conditions do not significantly damage or perturb the embryo, we found that it is possible to perform multiple rounds of electroporation. Researchers are going on in electroporation method for the treatment of HIV/ AIDS by killing $CD4^+$ T cells. In a recent research, electroporation can also be used for the regeneration of spinal cord. Electroporation could be achieved by using a glass electrode (tip diameter of 1–2 m, resistance of 10–15 MΩ) connected either to a conventional square pulse generator or a Grass stimulator.

CONCLUSION

In vitro EP has been established as a laboratory procedure in molecular biology for more than a decade. More recently, in vivo EP has proven to be a valuable tool in biomedical research, and its clinical feasibility and utility are already evident from human studies in oncology. Electroporation is potentially beneficial for practical applications in life sciences and biotechnology. In the past few years, various approaches have been developed to advance this field in a synergistic manner. In this review, we focus on recent advances in electroporation and its applications. Specifically, we also discuss current challenges and perspectives of electroporation for clinical applications. In the present time electroporation is widely used as a technology to provide better results. Electroporation is mostly used in DNA transfection, vascular therapy, gene therapy, in transdermal drug delivery system to enhance the permeability of drug into the skin. Electroporation is also used for many other beneficial applications. In cancer treatment electroporation plays a vital role in the study and treatment of tumors.These efforts have successfully used to widespread applications for studying hard-to-cure diseases (HIV and cancer), regenerative medicine (stem cells regeneration), and other diseases-related cells. To promote a wider use of electroporation in clinical fields, however, it may be required to enhance the efficiency as well as increase the ease of use. This approach will help researchers to promote a comprehensively integrative approach to advanced genetic and cellular research in life sciences.The sophisticated EP equipment now available automatically selects appropriate EP conditions, electronically monitors and records the actual treatment process, and controls the use of the disposable electrode applicators using smart chips.

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