IJPSR (2010), Vol. 1, Issue 12 (Review Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 11 July, 2010; received in revised form 07 October, 2010; accepted 13 November, 2010

NON DESTRUCTIVE METHOD FOR GENE AND DRUG DELIVERY IN LIVING CELL

K. B. Rathod*

K. B. Raval College of Pharmacy, Shertha, Kasturinagar, Gandhinagar, Gujarat, India

Keywords:

Electroporation,
Gene transfer,
Drug Delivery,
Gene therapy

Correspondence to Author:

K. B. Rathod

K. B. Raval College of Pharmacy, Shertha, Kasturinagar, Gandhinagar, Gujarat, India

ABSTRACT

Gene transfer represents a relatively new way for the treatment of rare genetic disorders by changing the expression of a person's genes. Electroporation is a mechanical method of gene transfer used to introduce polar molecules such as DNA, RNA and Peptides (Heparin, Insulin) into a host cell through the cell membrane. In this procedure, a large electric pulse temporarily disturbs the phospholipid bilayer, allowing molecules like DNA to pass into the cell. With careful control of the strength of the electrical field and the duration of the cell's exposure to it, the pores will reseal themselves after a short period of time. There are nine ways for gene transfer: 1. Electroporation; 2. Lipid mediated method; 3. Chemical mediated gene transfer; 4. Microinjection; 5. Biolistics; 6. Viral vectors; 7. Transformation; 8. Conjunction; 9. Gene Transfection enhanced by elevated temperature. Among a number of techniques using lipid, viruses or specialty reagents may be cytotoxic, labor intensive and costly while, Electroporation is a simple, inexpensive, safe, non toxic and non viral method. The application of electroporation to enhance transdermal delivery has opened up a new possibility to introduce larger molecules such as peptide hormones and vaccines as well as minigenes and RNAi etc. This review describes the method by which polar molecules are delivered to the cells. Other important issues such as the instrumentation, advantages and application of Electroporation are also addressed. Electroporation is non destructive method of gene and drug delivery.

INTRODUCTION: In the 1970s, scientists discovered that applying electrical pulses to a cell (in a lab situation) enabled dramatically increased uptake of a biological material into the cell. While widely used in research laboratories, it wasn't until the 1990s that the first research was undertaken to investigate potential direct applications of electroporation to humans in vivo. Today, Inovio commands a dominant position in intellectual property relating to human applications of electroporation. Inovio research has shown the potential utility of electroporation for human applications including oncology, gene therapy (including DNA-based immunotherapies and vaccines), cosmetic, vascular, transdermal, plant and ex vivo applications. In the past two decades, the mechanism and practical applications of electroporation (also termed electropermeabilization) have received increasing attention, particularly as a means of introducing a range of drugs, DNA, antibodies and plasmids into cells. Electroporation is also thought to play an active role in the treatment of ventricular fibrillation by defibrillation shock.

Electroporation involves a pulse of high voltage applied to protoplasts/cells/ tissues to make transient (temporary) pores in the plasma membrane which facilitates the uptake of Foreign DNA. The cells are placed in a solution containing DNA and subjected to electrical shocks to cause holes in the membranes. The foreign DNA fragments enter through the holes in to the cytoplasm and then to nucleus. It is also known as electropermeabilization. The phenomenon of electropermeabilization of cell membranes has been known for several decades, and has recently received increasing attention for the manipulation of cells and tissues [1-7.] Very early observations suggested that some type of "electrical breakdown" might occur in electrically stimulated membranes [8].

The stratum corneum (SC), the outermost layer of the skin, is the main barrier to transdermal transport. Electroporation has been used to deliver drugs and other molecules across the skin. The electrical resistance of the SC is between 5 and 25 kV/cm^2 [9] and has an electrical breakdown potential around 75–100 V [10]. The molecular weight cutoff has determined, using FITC-dextrans, to be around 10 kDa [11, 12].

Mechanism: Electroporation allows cellular introduction of large highly charged molecules such as DNA which would never passively diffuse across the hydrophobic bilayer core [13]. Electroporation is a multi-step process with several distinct phases [14]. First, a short electrical pulse must be applied. Typical parameters would be 300-400 mV for < 1 ms across the membrane. Upon application of this potential the membrane charges like a capacitor through the migration of ions from the surrounding solution. With careful control of the strength of the electrical field and the duration of the cell's exposure to it, the pores will reseal themselves after a short period of time **(Figure 1, 2).**

Instruments and Working Principle: Electroporation is based upon the use of short electrical impulses of high field strength. These impulses increase the permeability of cell membrane and facilitate entry of DNA molecules in to the cells, if the DNA is in direct contact with the membrane. The electroporation pulse is generated by discharging a capacitor across the electrodes in a specially designed electroporation chamber.Either a high voltage (1.5 kV) rectangular wave pulse of short duration or a low voltage (350V) pulse of long duration is used. The host cells and the molecules to be inserted into these cells are suspended in solution. When the first switch is closed, the capacitor charges up and stores a high voltage.

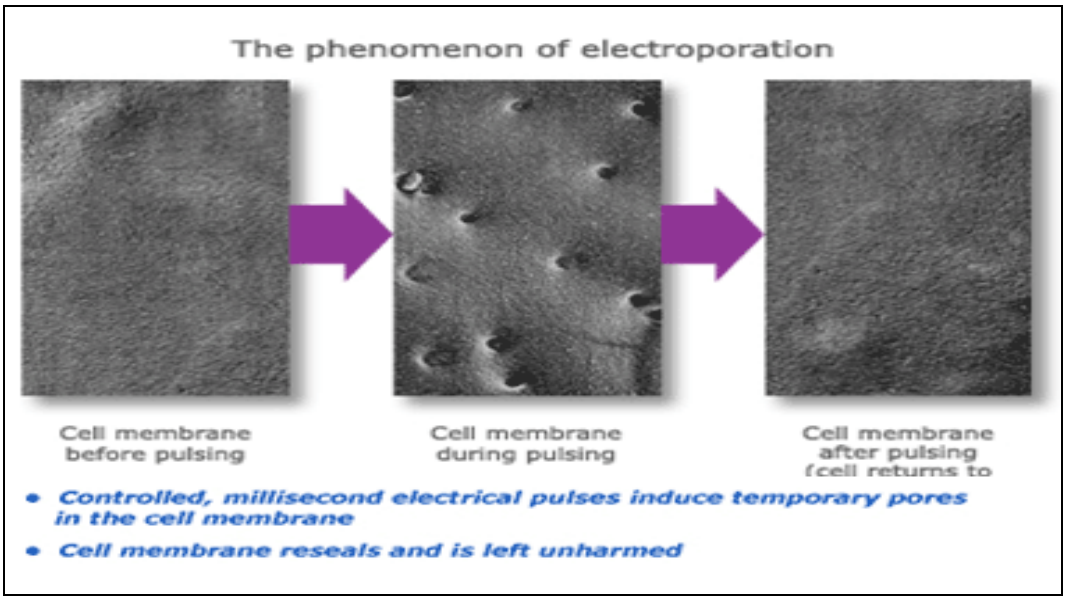

FIG. 1: MECHANISM OF ELECTROPORATION

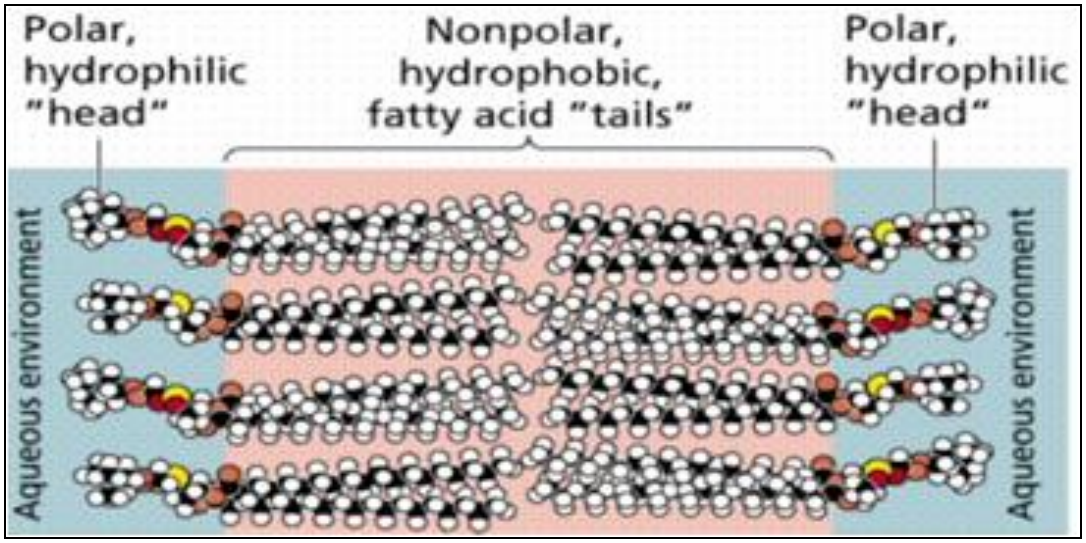

FIG. 2: DIAGRAM OF THE PHOSPHOLIPID BILAYER

When the second switch is closed, this voltage discharges through the liquid of the cell suspension. 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 phospholipid bilayer of the membrane and causes the formation of temporary aqueous pores. The electric pulses have two roles, to permeabilize the target cell plasma membrane and to transport the DNA towards or across the permeabilized membrane by electrophoresis. For efficient transfer, reversible undamaging target cell permeabilization is mandatory. There are essentially six major electroporation instruments from different companies presently on the market that are specifically designed to introduce macromolecules into cells. Such companies are Bio-Rad, BRL, Hoefer, BTX, Baekon, and Promega. The electroporation system designed by Promega is based on capacitor discharge.

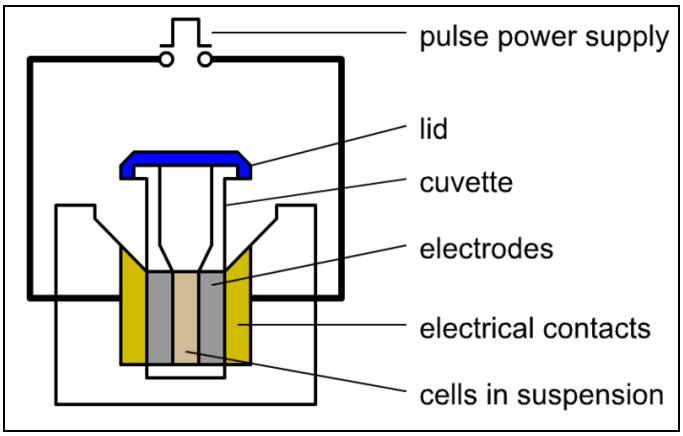

FIG. 3: DIAGRAM OF THE BASIC CIRCUIT SETUP OF THE ELECTROPORATION APPARATUS

Recent Electroporation Technology for Gene and Drug Transfer: Novel gene transfer technique for hard-to-transfect cells

1. **Microporation:** It is a unique electroporation technology using a pipette TIP as an electroporation space. In doing so, the uniform electric field is generated accompanying minimal heat generation, metal ion dissolution, pH variation and oxide formation. Great transfection efficiency and cell survival rate are only a few benefits from the Microporation technology.

- Efficient transfection of hard-to-transfect cells
- High cell viability and broad cell density
- No more cuvette, just a pipette tip
- Very fast method
- Low operation cost

FIG. 4: MICROPORATION TECHNOLOGY

2. **Electroporator for Gene Delivery:** The ECM 830 generator has been specifically designed for gene and drug delivery by electroporation in mammalian cells. Electroporation technology is widely used in experimental cancer research since it enhances the cellular uptake of various molecules. When a plasmid DNA is injected directly into a tumor lesion followed by electroporation, the localized electrical charge enhances its uptake into the tumors cells. Within the tumors, the plasmid DNA to produce specific proteins, this, in turn stimulates the immune system to generate antibodies against this tumor cells.

FIG. 5: ELECTROPORATOR FOR GENE DELIVERY

Application: The first biological use of electroporation--the formation of holes or pores in the cell membrane by high-voltage electric shock--was to induce cells to fuse via their plasma membranes. It was then found that the electropores could be used to introduce macromolecules into cells [15-18]. Electroporation can be used in many area of molecular biology and medical field include:

1. **DNA (Gene) Transfection or Transformation:**

- **Transformation of Bacteria by Electroporation:** It is now possible to transform gram-positive and gram-negative bacteria by electroporation. The efficiency of

transformation in E. coli is routinely 109/~g of input plasmid DNA, and can reach as high as 101°.

- **Transformation of Yeast by Electroporation:** As with bacteria, the small size of yeast cells compared to mammalian cells requires higher field strengths for efficient electroporation. For instance, yeast can be transformed after treatment with lithium acetate, to yield a transformation frequency of between 10^2 and 10^3 transformants per microgram of plasmid containing the yeast replication origin.

2. **Direct Transfer of Plasmids between Cells and Induced Cell Fusion: Figure 6** represent of plasmids containing a foreign DNA insert passing through temporary aqueous pores in the plasma membrane. The actual entry of DNA into the cell cannot be observed with a microscope [19].

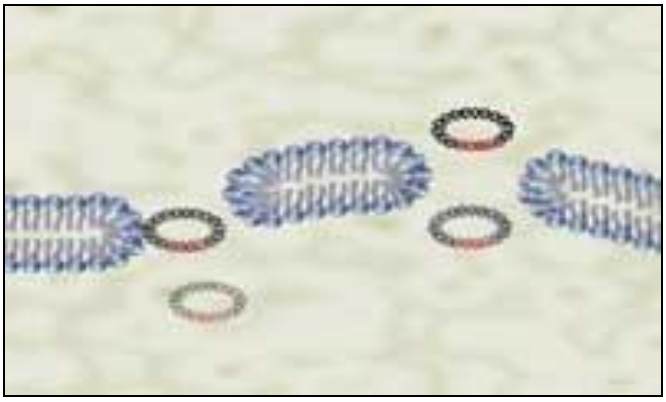

FIG. 6: DIRECT TRANSFER OF PLASMIDS BETWEEN CELLS AND INDUCED CELL FUSION

3. **Trans-dermal Drug Delivery** [20-26]: Although transdermal drug delivery has many potential advantages, the permeability of skin to macromolecules is extremely low. However, the application of short, high-voltage pulses to electroporate skin has recently been shown to make it reversibly permeable. A number of studies have demonstrated that electroporation-mediated transdermal delivery of peptides, polysaccharides, oligonucleotides and genes may be possible at clinically relevant rates, leading to the current commercial development of electroporation Techniques. Transdermal administration is attractive because it avoids degradation in the liver or gastrointestinal tract, but normal skin is impermeable to most compounds, especially macromolecules. By reversibly overcoming the skin's barrier properties, electroporation may provide a way to deliver these agents.

Electroporation has been used to transport several drugs through the skin in humans, including insulin, heparin, and the local anesthetic lidocaine. Studies also suggest that electroporation could be used to deliver compounds that would ameliorate skin aging, such as particular genes or Vitamin C.

Transdermal drug delivery via electroporation can be enhanced through the use of mild heat, alkaline solutions, and sodium dodecyl sulfate. Because the drug reservoir remains outside the body, transdermal drug delivery devices provide the opportunity to easily adjust the quantity and delivery rate of medications. Transdermal systems could be controlled by a miniature computer, which would allow for accurate dosing as needed by the patient. These systems might also include sensors that monitor blood levels of compounds, such as glucose in diabetics, and then adjust the release of a drug, such as insulin. These and other developments in transdermal drug delivery technologies hold promise for improving patient compliance by making drug administration effortless and painless.

4. **Cancer Tumor Electro chemotherapy and Gene Therapy:** Electroporation can be used to transfect bone marrow cells, which may then be reintroduced into a recipient animal [27]. There is good evidence that electroporated genes can recombine with their homologous host gene [28].

The resultant cell actually acquires the wild-type version in place of its mutant gene at the exact location in the chromosome that it normally should reside, thus reducing the potential mutagenetic effects of random insertion. The successful use of standard electroporation to introduce genes into the germline of mice can result in 1 in 1000 cells acquiring the exogenous DNA by recombination-insertion. *In vivo* electroporation-mediated gene therapy in large animals is gaining ground as one of the most important means for non-viral gene therapy.

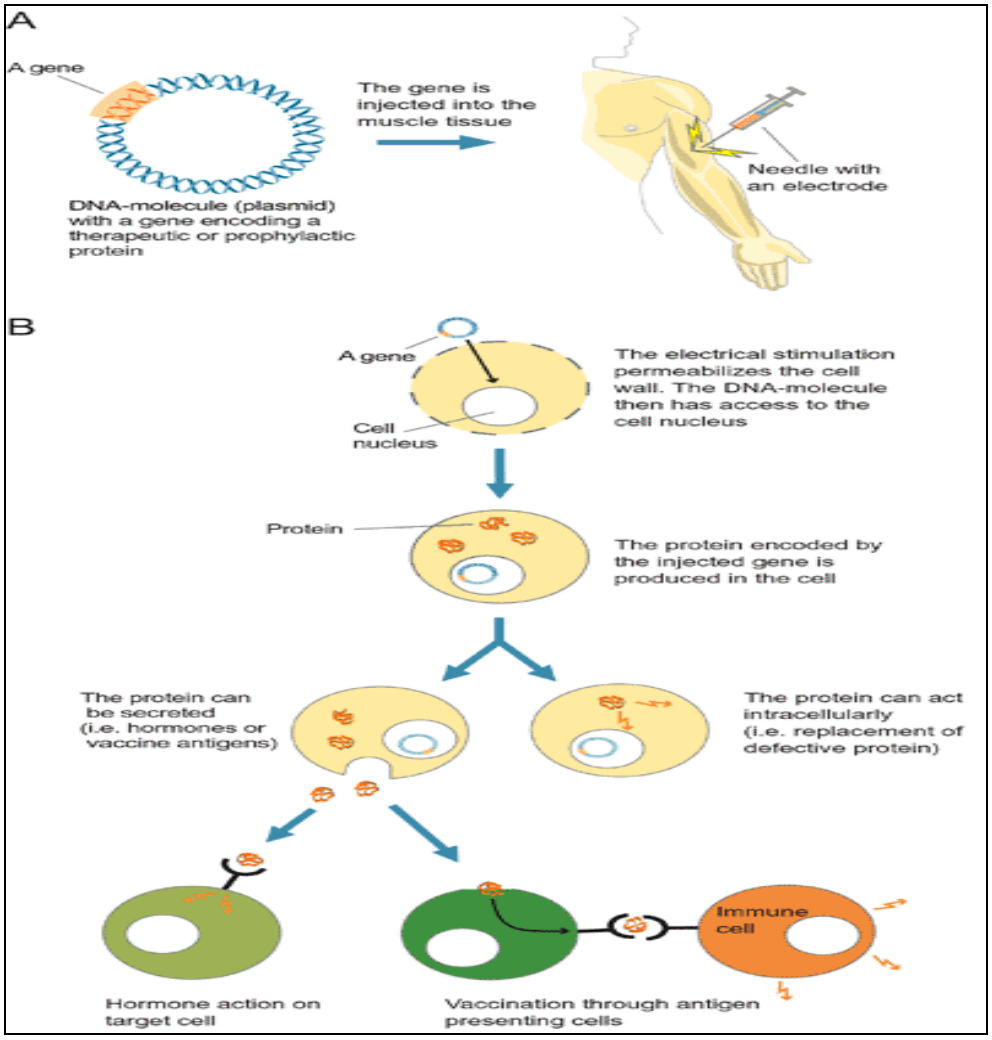

FIG. 7: DIAGRAM OF THE METHOD OF GENE THERAPY USING ELECTROPORATION

5. **DNA and drug Delivery** [29-31]: Electroporation is emerging as a preferred method for delivering DNA-based agents. Most drugs, and all genes being used for medicinal benefit, act on cellular machinery inside a cell in order to perform their intended function. Getting these agents through the cell membrane can be a significant challenge. Safely and effectively delivering DNA vaccines has been a persistent challenge to the advancement of this promising field of medical science. Electroporation involves the application of controlled, millisecond electrical pulses to create permeability in the cell membrane and enable dramatic uptake of a biological material previously injected into local tissue. Inovio's electroporation-based DNA delivery technology for DNA plasmid-based vaccines has displayed the following characteristics:

- Dramatically enhances cellular uptake of biological materials such as DNA vaccines
- Can induce significant immune responses, including antibody and T-cell responses
- Tolerable without anesthetic
- DNA plasmids used in conjunction with electroporation is easily, cost effectively manufactured, without manufacturing complexities
- Does not induce unwanted immune responses against the delivery mechanism, therefore it can be used for repeat administration of vaccines, e.g. for booster shots
- Electroporation itself works as an adjuvant to enhance the necessary "danger signals" that become detectable by the immune system.

Electroporation can significantly enhance the potency of DNA vaccines: Pre-clinical testing in large animal models has shown that Inovio's electroporation-based DNA delivery technology increased neutralizing antibody production more than 100 times. Pre-clinical testing in large animal models has shown that Inovio's delivery technology increased the breadth and magnitude of T-cell production from a multi-antigen DNA vaccine against HIV. The test resulted in an increase in potency of the DNA vaccine by over 200 times. [32]

Methods of gene transfer:
There are nine ways for gene transfer:

1. **Electroporation:** This method appears to provide a desirable balance of safety, efficiency, and cost effectiveness. The technique of electroporation-the formation of holes or pores in the cell membrane by high voltage electric shock-has found widespread application in biology.

2. **Lipid mediated method:** Liposomes are circular lipid molecules with an aqueous interior that can carry nucleic acids. Liposomes encapsulate the DNA fragments and then adher to the cell membranes and fuse with them to transfer DNA fragments. Thus, the DNA enters the cell and then to the nucleus. Lipofection is an also good technique used to transfer genes in bacterial, animal and plant cells.

3. **Chemical mediated gene transfer:** Chemicals like polyethylene glycol (PEG) and dextran sulphate induce DNA uptake into plant protoplasts. Calcium phosphate is also used to transfer DNA into cultured cells.

4. **Microinjection:** Microinjection where the DNA is directly injected into plant protoplasts or cells (specifically into the nucleus or cytoplasm) using fine tipped (0.5 - 1.0 micrometer diameter) glass needle or micropipette. This method of gene transfer is used to introduce DNA into large cells such as oocytes, eggs, and the cells of early embryo.

5. **Biolistics or gene gun:** The gene gun or Biolistics gun, which blasts microscopic DNA-vaccine-coated gold particles into the patient's skin, is thought to be an efficient method for administering DNA vaccines. However, its use in humans is generally associated with severe local pain, erythema lasting 2-4 weeks post delivery, and skin discoloration lasting up to 6 months. In some cases, skin necrosis is reported. [33] The microprojectile bombardment method was initially named as biolistics by its inventor Sanford (1988). Two types of plant tissue are commonly used for particle bombardment- Primary explants and the proliferating embryonic tissues. The challenge of these delivery methods is that the "carrier," i.e. virus, lipid, or gold particle, they use to transport the vaccine may introduce its own

unique challenges with respect to safety, utility, or manufacturability.

6. **Viral vectors:** Viral vectors (carriers) have been the most studied approach to intracellular DNA delivery. Leveraging the natural ability of viruses to insert and express a "genetic payload" in human cells, scientists modify a virus to carry beneficial genes such as a DNA vaccine. These viral vectors, containing their payload, are then injected by a syringe/needle in target tissue such as muscle. There are multiple issues with viral vectors that have plagued DNA vaccine developers using this approach:

- Viral vectors may insert their genes randomly into the target cell's chromosomes, risking disruption of genetic regulatory machinery and/or causing mutations that could lead to cancer [34].
- There are size constraints on the genetic payload that can be delivered [35].
- They may induce an unwanted immune response against themselves, making the patient resistant to subsequent vaccinations using the same viral vector. While these viral vectors may effectively deliver their payload during the prime or first vaccination, if booster (additional) vaccinations are required, the immune system might attack and remove the viral vector before it can deliver the vaccine it is carrying [36].
- Viral vectors can be difficult and expensive to develop and to manufacture in a controlled manner, and obtaining regulatory approval may be challenging [37].

The Pharmaceutical industry continues to invest significant resources to achieve the therapeutic profile, i.e. the right balance of safety and efficacy, necessary to enable viral vectors to play a role in facilitating DNA vaccine delivery. New therapeutic strategies are also being evaluated in which an alternative delivery method is used for prime applications of a vaccine and then a viral vector-based vaccine is used for the booster application of the vaccine. For example, Merck is conducting a clinical study to evaluate this prime-boost strategy with an electroporation-viral vector combination.

7. **Transformation:** This method is used for introducing foreign DNA into bacterial cells e.g. E. coli. The transformation frequency (the fraction of cell population that can be transferred) is very good in this method. e.g. the uptake of plasmid DNA by E. coli is carried out in ice cold $CaCl_2$ (0-50˚C) followed by heat shock treatment at 37-450˚C for about 90 sec. The transformation efficiency refers to the number of transformants per microgram of added DNA. The $CaCl_2$ breaks the cell wall at certain regions and binds the DNA to the cell surface.

8. **Conjunction:** It is a natural microbial recombination process and is used as a method for gene transfer. In conjunction, two live bacteria come together and the single stranded DNA is transferred via cytoplasmic bridges from the donor bacteria to the recipient bacteria.

9. **Gene Transfection enhanced by elevated temperature:** It is also method for gene transfer where Gene Transfection enhanced by elevated temperature.

Advantages of Electroporation:

- Versatility
- Efficiency
- Small Scale
- *In vivo*

Disadvantages of Electroporation:

- Cell Damage
- Nonspecific Transport

CONCLUSION: With the tremendous growth of biotechnology and the recent sequencing of the human genome, the demand for improved methods for the delivery of protein pharmaceuticals has resulted in the development of numerous technologies and companies focused on delivery methods. Electroporation is a simple, highly effective means of introducing cloned genes into a wide variety of cell types. It affords substantial benefits over alternative procedures, being easier to use, more efficient, and applicable to a larger number of different kinds of cells. As the parameters of electroporation become optimized, efficiency of DNA entry into the recipient cell may approach 100%.

In summary, even though electroporation has been known for approximately 35 years, it has only recently been applied to skin. In a relatively short time, the field has blossomed, fueled by a range of mechanistic studies and coupled with demonstrated transdermal delivery not only of small drugs but also of macromolecules including peptides, polysaccharides, oligonucleotides and genes. This review suggests that the delivery of protein- and gene-based drugs by electroporation could be an important vehicle for bringing more biotechnology products to the clinical market.

REFERENCES:

1. E. Neumann, A. Sowers and C. Jordan (eds.), Electroporation and electrofusion in cell biology, Plenum, New York, 1989.
2. T.Y. Tsong, Electroporation of cell membranes, Biophys. J., 60, (1991) 297-306.
3. D.C. Chang, B.M. Chassy. J.A. Saunders and A.E. Sowers. Guide to electroporation and electrofusion, Academic Press, 1992.
4. M. Blank, Electricity and Magnetism in Biology and Medicine, San Francisco Press, San Francisco, 1993.
5. J.C. Weaver, Electroporation: A general phenomenon for manipulating cells and tissue, J. Cell. Biochem., 5 1 (1993) 426-435.
6. S. Orlowski and L.M. Mir, Cell electropenneabilization: A new tool for biochemical and pharmacological studies, Biochim. Biophys.Acta, 1154 (1993) 51-63.
7. J.C. Weaver, Electroporation in cells and tissues: A biophysical phenomenon due to electromagnetic fields, Radio Sci., 30 (1995) 205-221.
8. R. Stimpfli, Reversible electrical breakdown of the excitable membrane of a Ranvier node, An. Acad. Brasil. Ciens., 30, (1958) 57-63.
9. J.S. DeNuzzio, B. Berner, Electrochemical and iontophoretic studies of human skin, J. Control. Release 11 (1990) 105– 112.
10. U. Pliquett, R. Langer, J.C. Weaver, et al.,Changes in the passive electrical properties of human stratum corneum due to electroporation, Biochim. Biophys. Acta 1239 (1995) 111 – 121.
11. D.A. Edwards, M.R. Prausnitz, R. Langer, J.C. Weaver, Analysis of enhanced transdermal transport by skin electroporation, J. Control.Release 34 (1995) 211 –221.
12. L. Langkjær, J. Brange, G.M. Grodsky, R.H. Guy, Iontophoresis of monomeric insulin analogues in vitro: effects of insulin charge and skin pretreatment, J. Control. Release 51 (1998) 47– 56.
13. Schaefer-Ridder M, Wang Y, Hofschneider PH (1982). "Gene transfer into mouse lyoma cells by electroporation in high electric fields". *Embo J.* 1 (7): 841–5.
14. J. C. Weaver and Y. A. Chizmadzhev."Theory of electroporation: A review "Biochemistry and Bioenergetics. 41. (1996) 135-160.
15. E. Neumann, M. Schaefer-Ridder, Y. Wang, and P. H. Hofschneider, *EMBO* J. 1, 841 (1982).
16. T. K. Wong and E. Neumann, *Biochem. Biophys. Res. Commun.* 107, 584 (1982).
17. H. Potter, L. Weir, and P. Leder, *Proc. Natl. Acad. Sci. U.S.A.* 81, 7161 (1984).
18. G. A. Evans, H. A. Ingraham, K. Lewis, K. Cunningharn, T. Seki, T. Moriuchi, H. C. Chang, J. Silver, and R. Hyman, *Proc. Natl. Acad. Sci. U.S.A.* 82, 5824 (1984).
19. Murthy SN, Sen A, Zhao YL, Hui SW. Temperature influences the postelectroporation permeability state of the skin. Journal of Pharmaceutical Sciences 2004; 93: 908-15.
20. Sen A, Daly ME, Hui SW. Transdermal insulin delivery using lipid enhanced electroporation. Biochimica *et* Biophysica Acta. 2002; 1564(1):5-8.
21. Prausnitz MR, Edelman ER, Gimm JA, Langer R, Weaver JC. Transdermal delivery of heparin by skin electroporation. Biotechnology 1995; 13:1205-1209.
22. Wallace MS, Ridgeway B, Jun E, Schulteis G, Rabussay D, Zhang L. Topical delivery of lidocaine in healthy volunteers by electroporation, electroincorporation, or iontophoresis: an evaluation of skin anesthesia. Regional Anesthesia and Pain Medicine 2001; 26:229-238.
23. Zhang L, Li L, Hoffmann GA, Hoffman RM. Depth-targeted efficient gene delivery and expression in the skin by pulsed electric fields: an approach to gene therapy of skin aging and other diseases. Biochemical and Biophysical Research Communications 1996; 220:633-636.

24. Zhang L, Lerner S, Rustrum WV, Hofmann GA. Electroporation-mediated topical delivery of vitamin C for cosmetic applications. Bioelectrochemistry and Bioenergetics 1999; 48:453-461.
25. Murthy SN, Sen A, Zhao YL, Hui SW. pH influences the postpulse permeability state of skin after electroporation. Journal of Controlled Release 2003; 93:49-57.
26. Mir LM, Bureau MF, Gehl J, Rangara R, Rouy D, Caillaud JM, *et al*. High-efficiency gene transfer into skeletal muscle mediated by electric pulses. Proc Natl Acad Sci USA 1999; 96: 262-7.
27. F. Toneguzzo and A. Keating, *Proc. Natl. Acad. Sci. U.S.A.* 83, 3496 (1986).
28. Chang, B. Chassy, J. A. Saunders, and A. E. Sowers, Eds.). Academic Press, San Diego, 1992.
29. Hofmann GA. Instrumentation and electrodes for in vivo electroporation. In: Methods in Molecular Medicine. Jaroszeski MJ, Heller R, Gilbert R. Eds. Humana Press, Totowa, NJ. 2000; 37-61.
30. Finnin B C, Morgan T M. Transdermal penetration enhancers: applications, limitations, and potential. J. Pharm. Sci. 1999; 88: 955-58.
31. Weaver JC. 1995. Electroporation Theory: Concepts and Mechanisms. In: Nickoloff JA, editor. Electroporation Protocols for Microorganisms. Totowa, New Jesey: Humana Press. p 1-26.
32. Mathiesen I. Electropermeabilization of skeletal muscle enhances gene transfer *in vivo*. Gene Ther 1999; 6: 508-14.
33. Fuller, DH et al., 2006. Preclinical and clinical progress of particle-mediated DNA vaccines for infectious diseases. Methods 40:86-97
34. Howe, SJ et al., 2008. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. J. Clin Invest. 118:3143-3150/
35. McCarty, DM. 2008. Self-complementary AAV vectors; advances and applications. Mol Ther. 16:1648-56.
36. Pantaleo, G., 2007. HIV-1 T-cell vaccines: evaluating the next step. The Lancet 8:82-83.
37. Kamen A and Henry O. 2004. Development and optimization of an adenovirus production process. J Gene Med S1:S184-92.

****