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Therapeutic Applications of Electroporation

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1. Introduction

Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals. Most common routes of administration include the preferred non-invasive peroral (through the mouth), topical (skin), transmucosal (nasal, buccal/sublingual, vaginal, ocular and rectal) and inhalation routes. Many medications such as peptide, antibody, vaccine and gene based drugs, in general may not be delivered using these routes because they might be susceptible to enzymatic degradation or cannot be absorbed into the systemic circulation efficiently due to molecular size, to be therapeutically effective. For this reason such drugs have to be delivered by injection. For example, many immunizations are based on the delivery of protein drugs and are often done by injection. Current effort in the area of drug delivery include the development of targeted delivery in which the drug is only active in the target area of the body (for example, in cancerous tissues) and sustained release formulations in which the drug is released over a period of time in a controlled manner from a formulation Cemaẑar *et al.* (1998); Serŝa (2000); Serŝa *et al.* (1993). This is achieved by combining phenomenon of electroporation with the application of drugs.

This makes the area of drug delivery a study in which experts from most scientific discipline can make a significant contribution. To understand the barriers to drug delivery, it is useful to consider anatomical structures at a length scale suitable for variety of structures: a cell, a tissue, a muscle, organ. Better medical treatments may not always require stronger medicinal drugs. A better effect could be achieved by an effective method of administration. Often differences in the mode of drug administration produce substantial changes in drug concentration requirement, and thus can affect the unnecessary side effects of some of the drugs in favor of the patient. A good example of this is electrochemotherapy. The major disadvantage of clinically established chemotherapeutic drugs is their lack of selectivity of tumor cells. Therefore for a noticeable antitumor effect, high doses of the chemotherapeutic drugs are needed, which often cause systemic toxicity leading to severe side effects Serŝa *et al.* (1993). In cancer chemotherapy, some drugs do not exhibit anti-tumour effects because of impeded transport through the cell membrane Miklavĉiĉ and Kotnik (2004). To overcome this difficulty, a number of new approaches for drug delivery systems have been attempted. One of the approaches to better drug delivery is by making it topical and more effective at the tumor site with the use of electric field . This is electrochemotherapy. In this article a brief overview of electroporation and its use in electrotherapy is discussed.

2. Electrochemotherapy (ECT)

The combined use of chemotherapeutic drugs alongwith electroporation caused due to application of electric pulses is known as electrochemotherapy and is useful for local tumour control. Bleomycin and cisplatin which are commonly used drugs for chemotherapy have proven to be much more effective in electrochemotherapy than in standard chemotherapy when applied to tumour cell lines in vitro, as well as in vivo on tumours in mice Mir *et al.* (1991; 1995); Serŝa *et al.* (1995). Clinical trials have been carried out with encouraging results Glass *et al.* (1996); Gothelf *et al.* (2003); Kranjc *et al.* (2005); Serŝa *et al.* (2000); Snoj *et al.* (2005); Tozon *et al.* (2005). Especially, bleomycin has been reported to have shown a 700-fold increased cytotoxicity when used in ECT Cemažar *et al.* (1998); Serŝa (2000).This helps to achieve a substantial anti-tumour effect with a small amount of drug, that limits its side effects Serŝa *et al.* (1993).

2.1 Electroporation

It is possible to induce the formation of hole in a cell membrane by applying a sufficiently strong electric field pulse. This is known as electroporation. The effect is reversible when the cell membrane is temporarily permeated. Irreversible electroporation occurs when the cell membrane poration is of such a nature as to induce cell death. Polarization is one of the basic mechanisms of interactions of membranes with electric fields, leading to electroporation and related phenomenons of dielectrophoresis Neumann (1989); Pohl (1978) and electrofusion Neumann (1989); Zimmermann (1982).

2.1.1 Polarization of membranes

Polarization of membranes underlies their destabilization. Polarization is due to restricted motion of charges: electric fields exert forces on charges. These charges can either move if they are free (material is conductive) or accumulate if they are limited in their movement. This charge redistribution in a particular limited space leads to polarization. Figure 1 shows polarization of a single cell due to restriction by the cell membrane to the motion of ions.

2.2 Electric field interaction with polarized membranes and pore formation

The interaction of external electric field with the polarized membranes results in forces which can induce motions inside particles. This motion can result in structural rearrangement or fracture in the material. This can subsequently lead to electroporation and related phenomenon in case of cell membranes Dimitrov (1995); Pohl (1978). Membranes have low polarizability (relative dielectric constant about 5) and low conductivity (3×10^{-7} S/m) Kotnik *et al.* (1998). The cell membrane is generally bounded (externally and internally) by a medium of high dielectric constant (about 80) and a high conductivity (about 1.2 S/m). Application of external fields leads to accumulation of charge at the membrane surfaces; this creates an electric field inside the membrane that is much stronger than the surrounding field. The polarized membrane interacts with this field, resulting in structural rearrangements which can cause membrane poration.

It soon became apparent that a field-induced permeability increase is transient in nature although long-lived compared with the field duration. The term 'electropermeabilization' was used to explain the occurrence of permeability changes introduced by electrical impulses in vesicular membranes Neumann and Rosenheck (1972). It was later shown that the electric

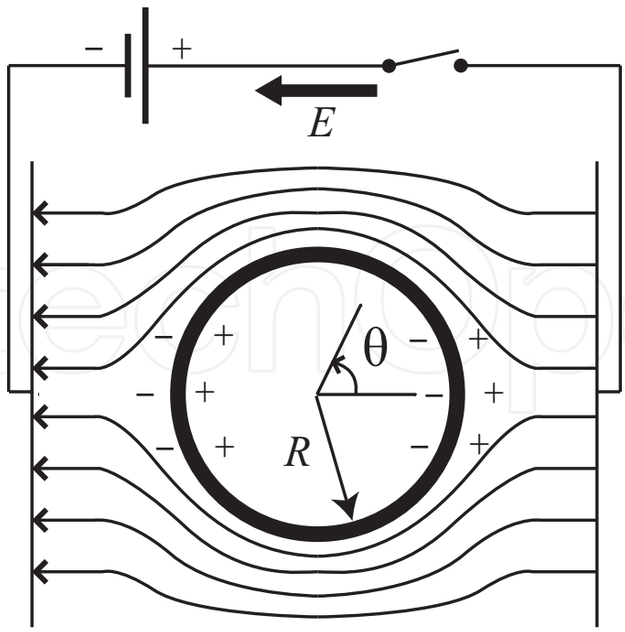

Fig. 1. Spherical particle in electric field, E is the electric field (Adapted from Chang *et al.* (1992); Dimitrov (1995)).

field induced change was transient Rosenheck *et al.* (1975) . The resistance changes in the membrane were attributed to dielectric breakdown Zimmermann *et al.* (1973).
Subsequent studies showed that the cell membranes of pulse treated cells were permeable to molecules of a size smaller than a certain limit, suggesting the creation of a porous membrane structure Kinosita and Tsong (1977b); Neumann and Rosenheck (1972); Zimmermann *et al.* (1973). It was also found that under appropriate conditions, the cells could recover, which implied that these electropores were resealable and could be induced without permanent damage to the cell Zimmermann *et al.* (1980), and the cytoplasmic macromolecular contents could be retained Kinosita and Tsong (1977a;b). Since then, a number of research groups have studied mechanisms of pore formation and detailed characteristics of the cell membranes modified by electric fields Abidor *et al.* (1979); Chernomordik *et al.* (1983); Glaser *et al.* (1988); Schwister and Deuticke (1985).
However, the pores themselves were not observed until the invention of rapid freezing electron microscopy in the 1990s. Chang et.al Chang and Reese (1990) were the first to observe them. Other aspects of electroporation, for example, vizualization of transmembrane potential and its evolution in space and time, resealing of pores and asymmetry in permeability of porated cells (sea urchin egg and liposomes) with the help of an optical microscope, were also reported Hibino *et al.* (1993); Kinosita *et al.* (1992). These microscopes have a time resolution of sub-microseconds suitable for studying electroporation.

2.2.1 Types of pores

The pores are assumed to be hydrophobic or hydrophilic. The hydrophobic pores, as shown in Figure 2a Abidor *et al.* (1979); Glaser *et al.* (1988); Neu and Krassowska (1999), are simply gaps in the lipid bilayer of the membrane, formed as a result of thermal fluctuations.

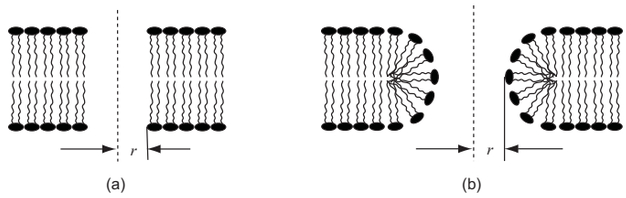

Fig. 2. Types of electropores: (a) Hydrophobic (nonconduting pore), (b) Hydrophilic pore (conducting pore).

The primary pores that participate in electrical behaviour and molecular transport are thought to be hydrophilic pores, with a minimum radius of about 1 nm, and a reasonable probability of various pore sizes much larger Weaver (1993). The 'hydrophilic' or 'inverted pores,' as shown in Figure 2b, have their walls lined with the water-attracting heads of lipid molecules. Hence, the hydrophilic pores allow the passage of water-soluble substances, such as ions, while the hydrophobic pores do not.

2.3 Facts about electrochemotherapy

Due to the availability of these electropores, electroporation can and has been used to deliver a variety of molecules for the purpose of DNA transfer, anesthesia, cosmetics, vaccination and chemotherapy. We discuss the electrochemotherapy details and results in brief as below.

1. Many studies reported that with belomycin doses far below toxicity, antitumour effectiveness of electrochemotherapy induced good responses of the tumours including tumour cures Serŝa (2000).
2. It has also been found that some tumours are more sensintive to one drug than to another used in electrochemotherapy Serŝa (2000).
3. Not all tumours have equal level of sensitivity to electrochemotherapy with bleomycin, but all tumor types (e,g., breast, colon, bladder, renal cell, malignant melanoma, basal cell carcinoma) have shown a response to electrochemotherapy Gehl (2008); Serŝa (2000).
4. Electrochemotherapy with bleomycin was performed on tumours in internal organs (brains and livers in rats) Serŝa (2000).
5. Complete eradication of treated nodules occurs in approximately 75% of the cases, and at least a partial remission occurs in 85-90% of the treated patients Gehl (2008).).
6. Mostly square pulses of duration 100 us, with electric field intensity of 1300V/cm or 1500V/cm, repetition frequency of 1 Hz is used. With higher amplitudes more cells in the tumour are permeabilized Gehl (2008).
7. Permeabilization of tumour cells is also dependent on the number of electric pulses, with eight electric pulses found optimum Jaroszeski *et al.* (2000).
8. Antitumour effectivenss is dependent on drug concentration in the tumour during application of electric pulses, a 3-minute interval between the treatments is optimal Jaroszeski *et al.* (2000).
9. The second most useful drug for electrotherapy has been found to be cisplatin. This is one of the drugs that induces resistance in cells, often early in the course of chemotherapy treatments. Electroporation has demonstrated itself to overcome this resistance of cells to cisplatin, at least to some degree Serŝa (2000).

10. Other attempts to determine whether other drugs would be effective in electrochemotherapy protocol in vivo do not prove to be good candidates because of their lipophilicity (being soluble in fat solvents) Serŝa (2000).
11. Achieving optimal electroporation during therapy without the need for repetitive treatment is an issue yet, however several ways of obtaining this are under research e.g. optimization of electric field by proper choice of value, number and duration of pulses and type of electric field, needle electrode design, rotating these needles between pulses Jaroszeski *et al.* (2000); Serŝa *et al.* (1993).
12. Impact of electrochemotherapy on the formation of metastates is yet to be established with preclinical and clinical studies, however studies have shown decreased number of metastases in rats Orlowski *et al.* (1998).

2.4 Noteworthy treatments of electroporation

There are many other special noteworthy applications of electrochemotherapy/ electroporation worth mentioning separately as below:

1. Treatment of human pancreatic tumours: cancers of pancreas is currently the fifth leading cause of cancer related deaths with a very low five year survival in the United States Jaroszeski *et al.* (2000). Since it is hard to detect in the early stages, it becomes difficult to treat. Conventional chemotherapeutic agents have not been vey effective for human pancreatic cancers Talele *et al.* (1991). A novel cancer treatment that uses a single intratumoral injection of bleomycin followed by application of the tumour site with square wave pulses has produced a large percentage of cures and a good number of partial regression in many different forms of cancers e.g. in human larynx Nanda *et al.* (1998b), human pancreas Dev *et al.* (1997); Nanda *et al.* (1998a).
2. Electrofusion (EF): Under appropriate physical conditions, delivery of electric pulses can lead to membrane fusion in close-contact adjacent cells. EF results in the encapsulation of both original cellsíntracellular material within a single enclosed membrane and can be used to produce genetic hybrids or hybridomas Zimmermann (1982). Hybridomas are hybrid cells produced by the fusion of an antibody secreting stimulated B-lymphocytes, with a tumour cell that grows well in culture. The hybridoma is then able to continue to grow in culture, and a large amount of specific desired antibodies can be recovered after processing. Electrofusion has proved to be a successful approach in the production of vaccines Orentas *et al.* (2001); Scott-Taylor *et al.* (2000), antibodies Schmidt *et al.* (2001), and reconstructed embryos in mammalian cloning Gaynor *et al.* (2005).
3. Transdermal drug delivery (TDD): Application of high-voltage pulses to the skin allows a large increase in induced ionic and molecular transport across the skin barrier Prausnitz *et al.* (1993). This has been applied for transdermal delivery of drugs, such as metoprolol Vanbever *et al.* (1994), and also works for larger molecules, for example, DNA oligonucleotides Vanbever *et al.* (1994).
4. Electroinsertion (EI): Another application of electroporation is insertion of molecules into the cell membrane. As the electric field induced membrane pores reseal, they entrap some of the transported molecules. Experiments on electroinsertion suggest the possibility of using the process to study certain physiological properties of these cells and understanding aspects of the lipid-protein interactions of the cell plasma membrane Mouneimne *et al.* (1992).

2.5 Known side effects of electrochemotherapy

1. During electrochemotherapy, the pulse delivery is usually painful for patients due to a a muscle contraction. Generally, a number of electric pulses are delivered, with a repetition frequncy of 1 Hz, which results in equal number of individual sensations and muscle contractions Zupanic *et al.* (2007). To reduce the number of individual muscle conractions, use of pulse frequncy larger than the frequncy of tetanic contraction has been suggested Miklavcic *et al.* (2005). It has been reported that increasing the pulse repetition frequncy to 5 kHz lowers the number of contractions, whereas clinical effectiveness remains same as that achieved by 1 Hz Marty *et al.* (2006); Snoj *et al.* (2007).
2. Just after the electric pulse delivery to the tumour nodule on the mice flanks, it is regularly observed a transient paralysis of the hind legs of the treated mouse, which lasts less than one minute and is always totally reversible Orlowski and Mir (2000).
3. Few days after electrochemotherapy, small scabs are often observed on the skin just at the level of electrode application. They always heal after a few days Orlowski and Mir (2000).
4. The delivery of electric pulses to tumours induces changes (reduced) in tumour blood flow. These changes have been observed to be sensitive to the frequency of applied electric field. The immediate reduction in tumour blood flow at 5 kHz was higher than the reduction at 1 Hz during the initial perid following pulse delivery however at longer times, the 5 kHz frequency had effects on tumour bood flow comparable to those abserved at 1 Hz Raeisi *et al.* (2010). Reduction in tumour blood flow may result in trapping of the drug in tumours, thus providing a longer time for the drug to act by decrease the drug washout from the tumour Mir (2006).
5. That antitumour effectiveness of electrochemotherapy is not only due to increased cytotoxicity of the drugs due to electroporation of tumour cells, but also due to reduced tumour blood flow and oxygenation Sersa *et al.* (2008).

3. Electrogenetherapy

Application of electroporation for transfer of DNA into cells to effect some form of gene therapy, often referred to as electrogenetransfection/electrogenetherapy, is currently being applied in some clinical trials. It is presently considered to have large potential as a non-viral method to deliver genetic material into cells, the process aimed at correcting genetic diseases Budak-Alpdogan *et al.* (2005). The genes being coiled up need a larger electropore for a longer time in order for it to enter the cells. Numerical modelling is useful to establish appropriate parameters to achieve this Krassowska and Filev (2007); Talele and Gaynor (2007); Talele *et al.* (2010). We have seen that due to electroporation the cells can be permeabilized such that the barrier function of the membrane is instantaneously compromised. During this time, genetic material may travel across the membrane. A successful gene transfer process is the one where the electrical and biological conditions of the cell are such that the barrier function of the cell membrane is rapidly restored for a cell survival. This process is termed a electrogenetransfer and when used for therapeutic purpose, electrogenetherapy. For gene therapy to be successful, the gene must be transferred efficiently to target cell without the cell damaging side effects. Most common method for gene transfer in the literature is the viral vector method to attach the gene of interest to enter in the target cell. This method may have detrimental effects of the virus Feuerbach and Crystal (1996) and thus alternative methods of

gene transfer are necessary. The electrogenetherapy seems to be the most promising one since the side effects are close to none.

One very obvious fact is that the intake of genetic material by an electroporated cell is affected by the extent of cell membrane permeabilization. This can be dependent on several parameters like cell diameter, cell membrane thickness and capacitance, internal and external conductivities, the electric pulse parameters used for electroporating, the time duration they are used for and so on. Many of these parameters have an interdependent and a non linear effect on the end result and need complex mathematical use to be explained in full details. Without getting into these details, I would like to mention some prominent simple to understand comments about electrogenetherapy.

1. Once the cell is permeabilized by a pulse, more DNA enters the cells during the next pulse of lower field strength Sukharev *et al.* (1992). Multiple pulses and AC pulses seem to have better results Chang *et al.* (1991).
2. Adding the DNA immediately after the pulse usually results in a much lower transfection efficiency compared to adding DNA before the pulse Andreason and Evans (1989).
3. For a given pore forming pulse electric field, the transfection efficiency depends more on the total length of the pulses than of the time span when cells remain permeable, which suggests that uptake of DNA adsorbed on cell surfaces would also contribute to this efficiency Nickoloff and Reynolds (1992).
4. Other physical parameters such as geometry and concentration of DNA are also important e.g. bigger size DNA would need bigger pores on the cell wall and thus would be hard to enter Nickoloff and Reynolds (1992).
5. The transfection efficiency decreases with increasing gap between repeating pulses Chang *et al.* (1991). This also suggests that DNA is collected on the cell surface for subsequent push through the electropores.
6. If the electro pores are too large that cell membrane is unable to reseal then the cell dies.
7. Another reason the cells may not survive is the osmotic swelling. This is due to selective permeability of the membrane after electroporation. Once electroporated, the intracellular cell material molecules being large, these molecules cannot escape outside, however the small ions from outside can enter in, causing the cell to swell leading to bursting and death Baker and Knight (1983).

4. Conclusion

Electroporation may be widely used as a cancer treatment in near future with advantages of low toxicity and being topical and more effective at the tumor site. Newer drugs suitable for various types of cancers and an optimum methodology of application of the electric field is under extensive research. Electroporation may also be the popular phenomenon used for genetherapy without use of viral vectors.

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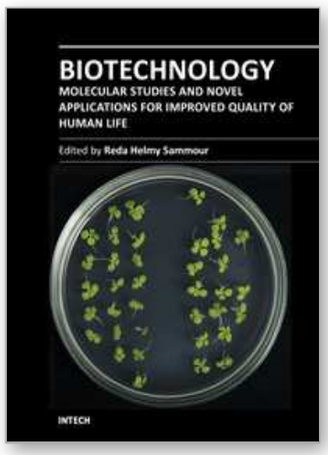

Biotechnology - Molecular Studies and Novel Applications for Improved Quality of Human Life

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This book deals with the importance of application of molecular biology as an approach of biotechnology for improvement of the quality of human life. One of the interesting topics in this field, is the identification of the organisms that produce bioactive secondary metabolites. It also discusses how to structure a plan for use and preservation of those species that represent a potential source for new drug development, especially those obtained from bacteria. The book also introduces some novel applications of biotechnology, such as therapeutic applications of electroporation, improving quality and microbial safety of fresh-cut vegetables, producing synthetic PEG hydro gels to be used as an extra cellular matrix mimics for tissue engineering applications, and other interesting applications.

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