Electroporation in Cancer Treatment
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Abstract—This paper provides a summary of the role of electroporation in experimental and accepted cancer treatments, including electrochemotherapy, gene electrotransfer, and tumor ablation with irreversible electroporation.

I. Introduction
Electroporation is the process of increasing cell membrane permeability to ions and molecules by exposing the cell to short (micro- to millisecond) high voltage electric pulses. This increase in permeability can be used to enhance the penetration of drugs or to introduce DNA plasmids, and in the case of irreversible electroporation, to destroy undesirable cells.[1] Methods of electroporation are used in microbiology, biotechnology, and medicine. Following an examination of the electrical and physical mechanism behind electroporation, the research and applications of this process in chemotherapy, gene therapy, and tumor ablation will be summarized and discussed.

II. Physical Mechanism
The cell membrane, which separates the interior of a cell from its external environment, consists of a lipid bilayer with embedded proteins. The lipid bilayer is approximately d = 5 nm thick, and is a good conductor, exhibiting an averaged resistivity in the order of 10^9 Ωcm.[10] The lipid bilayer has a relative dielectric gradient that varies from 1-2 at the hydrophobic core, to 4-25 at the outer/inner facing head regions.[11] For our analysis we will consider a simplified lipid bilayer dielectric constant of k = 5. The cell membrane has a capacitance, C_m, which is directly proportional to the membrane surface area (A) and the dielectric properties of the membrane, such that

\[ C_m = \frac{k \varepsilon_0 A}{d}. \]

Using our assumptions, we find that the capacitance per unit area is in the order of 10^{-6} F/cm^2. The cell membrane can be modeled as an RC circuit, with R_m as the membrane resistance, C_m as the membrane capacitance, and resting transmembrane voltage V. With applications of high voltage across the lipid bilayer at times greater than the time constant \( \tau = R_m C_m \), there will be a buildup of transmembrane voltage in addition to the resting voltage V. Once the transmembrane voltage exceeds a few hundred millivolts, rearrangement of the bilayer structure begins, and the membrane acquires new conduction pathways.[10] The rearrangement of lipids results in nm-scale holes in the cells membrane, occurring at locations of deformation in the bilayer structure.[16] In our simplified model, we note that with more channels open between the lipid layers, the resistance R_m decreases. The critical voltage level at which pores begin to form in the cell membrane is considered the threshold for electroporation.

It is important to note that this simple RC circuit model is linear and ignores several functions that contribute to the non-linear electromechanical nature of electroporation and the cell membrane in general. A more realistic model could break the
resistance down into series and shunt resistances, and could also consider the resistance of cytoplasm within the cell. These resistances are functions of cell size, protein distribution, existing ion channels, and deformations in the bilayer structure. Additionally, the cell membrane capacitance is a function of cell size and can vary depending on differences in the dielectric gradient of the lipid bilayer.[15] Non-linear single cell electroporation models have been developed on microfluidic chips, which allow for the prediction of critical voltages for different cell sizes.[12] This type of model is built upon numerical analysis and is beyond the scope of this paper, however is important for the refinement of electroporation treatments. With more accurate prediction of electroporation thresholds for a given cell line, treatments using reversible electroporation can further reduce undesirable cell damage and provide a more targeted response.

### III. Electrochemotherapy

The increased permeability of cell membranes due to electroporation allows otherwise impermeable molecules to be introduced to cells. Anti-cancer drugs can be more efficiently delivered to tumor cells with this increase in permeability in the process known as electrochemotherapy. Cutaneous and subcutaneous tumors have been treated using electrochemotherapy with drugs bleomycin and cisplatin, to a response rate of 80%.[24,25] Tumor mass reduction has been achieved with electrochemotherapy in a shorter time frame than standard chemotherapy.[24] One of the biggest advantages of delivering chemotherapy drugs via electroporation is a reduction in the effective dosage required. A 2018 study on 28 patients receiving electrochemotherapy for head/neck skin cancer gave one group (n = 12, 24 lesions) a reduced bleomycin dose (10,000 IU/m²) and the other group (n = 16, 28 lesions) a standard bleomycin dose (15,000 IU/m²). The results showed complete tumor response from both groups, indicating that a reduced dosage can be delivered when using electrochemotherapy compared to traditional methods.[34] Electrochemotherapy is used extensively in veterinary oncology for tumors in dogs, cats and horses.[31] The process has been approved for use on cutaneous and subcutaneous tumors in human patients in several countries in the EU, as well as the United States.[32] Treatments of deep-seated tumors, such as in bones or internal organs, are still in various phases of research and approval. The most promising of these is the treatment of liver tumors, which has shown so far to be safe and successful in several clinical trials.[35,36,37]

### IV. Gene Therapy

In gene therapy, plasmid DNA or short interfering RNA is introduced to cells in order to replace a mutated gene with a functional one. A common vector for introduction of DNA to cells is viruses, which copy their genetic material into the host cell’s genome. Therapeutic DNA or RNA is substituted in a virus’ genetic material and introduced to a living organism in vivo or to cell cultures in vitro. A potential complication from this delivery method is immune rejection response, of which one human trial patient has died.[3] Non-viral transfer methods are advantageous to gene therapy from a safety standpoint as they show lower host immunogenicity. Electroporation allows for the transfer of DNA or RNA into cells in the process called gene electrotransfer.

Gene electrotransfer was first described in a 1982 study, showing that linear and circular plasmid DNA could be introduced into mouse fibroblast
cells in vitro alongside 5 μs pulses, with an optimal field strength of 8 kV/cm.\textsuperscript{[26]} The first in vivo gene electrotransfer experiment was performed in 1991, in which plasmid DNA was introduced to mice skin cells via electroporation with 100 μs pulses at 600 V/cm.\textsuperscript{[4]} Note the much lower electroporation field strength threshold in mice skin cells, as well as the much longer pulse width used, in comparison to in vitro fibroblast cells. This is due in part to the difference in cell diameter of fibroblast cells (5-10 μm) and skin cells (30 μm).\textsuperscript{[27]} Temperature and cell shape are other factors in differing electroporation thresholds.

The first human trial of gene transfer via electroporation occurred in 2007, testing the introduction of therapeutic DNA as a treatment for metastatic melanoma. Six pulses at a field strength of 1.3 kV/cm and pulse width of 100 μs were delivered across six needle electrodes inserted into the tumor, with plasmid DNA being delivered through a central needle. 42% of patients in this trial showed disease stabilization or partial response, and 10% showed complete regression of all metastases.\textsuperscript{[5]} The largest adverse effect of this trial was localized pain around the application area.

The field of gene therapy research has faced several problems over the past 20 years, including the deaths of three patients receiving gene therapy treatment in medical trials.\textsuperscript{[3,29,30]} Gene therapy research also faces speculation and ethical concerns of genetic modification of humans. Despite these hurdles, several gene therapy products have been approved for use by the FDA, such as LUXTURNA, a treatment for patients with a rare form of inherited vision loss.\textsuperscript{[2]} Research into non-viral genetic vectors like the described gene electrotransfer method will hopefully give future promise to safer and more efficient genetic therapy treatments.

V. IRREVERSIBLE ELECTROPORATION

Pulses delivering a great enough field strength to a cell can cause irreversible electroporation, a state in which the pores in the cell membrane are opened permanently. This causes the cell to either leak or expand and die by apoptosis.\textsuperscript{[28]} It is generally accepted that as the applied electric field increases, the greater the disruption of lipid head groups, which increases the number of pores occurring on the cell membrane.\textsuperscript{[18]} However, timing of pulse delivery can also play a role in the effectiveness of cell death by electroporation. Simulations show that a time-window exists after delivery of an electric pulse at which pores reach a maximum radius before closing. A second pulse delivered at this time-window can result in more efficient destruction of the cell by forcing the pores to remain open.\textsuperscript{[17]} This study found that hydrophilic pores can form in 5 - 10 ns, with a much longer period needed for the resealing of pores. Data from this study lines up with other theoretical results that show pore formation occurs within 10 ns\textsuperscript{[19]} while resealing occurs in a time-frame of seconds.\textsuperscript{[20]}

Tumor treatment *in vivo* using irreversible electroporation was first described successfully in a 2007 study on mice. Plate electrodes delivered 80 pulses at 0.3 Hz with 100 μs pulse width across cutaneous tumor locations. Voltage was adjusted using electrode distance measurements to establish an electric field strength of 2500 V/cm. These conditions resulted in 12 of 13 mice showing complete regression. Heating effects due to pulse delivery were shown to be insignificant in this study.\textsuperscript{[8]}
The treatment of a brain tumor in a canine model was investigated in a 2011 study. Delivery of electroporation pulses was planned in this case using 3D reconstructed MRI imagery of the tumor. Four sets of 20 pulses with 50 μs pulse width were delivered across the tumor mass, synchronized with the dog’s heartbeat to prevent arrhythmia. The distance between electrodes was 5 mm and the voltage across the electrodes was 625 V. The conductivity of brain tumor cells was shown to increase between 3.42 and 3.67 times the baseline conductivity during electroporation. Three days after treatment, MRI’s revealed a 75% reduction in tumor mass. Following the treatment, the dog received whole-brain radiotherapy for 4 weeks, and after 4 months showed complete remission of the tumor. This study highlights the use of reconstructed MRI images to plan treatment, and the use of electroporation treatment alongside whole-brain radiotherapy.\[13\]

Irreversible electroporation treatments on human patients have been performed on kidney,\[21\] liver, lung,\[14\] and prostate\[22\] tumors. In 2012, John Hopkins surgeons began using irreversible electroporation in their treatment of pancreatic cancer. This treatment is used in cases where thermal ablation or surgical removal of tumor tissue is impossible, usually because of tumor location close to major blood vessels or bile ducts.\[9\] The local environment and tissue properties around a tumor can change the pulse voltage needed for tumor cell damage or death. For example, kidneys are susceptible to irregular ablation zones due to the increased conductivity of urine. The presence of metal, such as in biliary stents, can also result in variances in the ablation zone.\[23\]

VI. SAFETY OF ELECTROPORATION TREATMENTS

Electroporation as a delivery method of drugs or genes has been shown to be safe, with most studies reporting the largest adverse effect as localized pain around the application area.\[6\] The small pulse width, use of direct current, small electrode distances, and application on locations far from the heart contribute to the belief that electroporation pulses are generally safe. Real-time computer controlled adjustment of voltage during pulse delivery can help ensure electroporation remains reversible, thus minimizing cell death.\[7\]

The high voltages used in electroporation techniques could be cause for concern, especially in irreversible electroporation treatment where voltages can exceed 3 kV. The delivery of electric pulses during the “vulnerable period” of a heart beat could induce fibrillation, with greater chance in patients with functional or structural heart abnormalities, or in the elderly. Research has been conducted into time-controlled delivery of pulses in order to increase the safety of electroporation treatments.\[6\] A 2011 non-randomized study into the safety of irreversible electroporation in kidney, liver, and lung tumor ablation reported transient ventricular arrhythmia in four out of eight patients receiving non-synchronized electroporation pulses. The subsequent 30 patients in this study received synchronized treatments and only 2 further arrhythmias occurred. The conclusion of this study was that irreversible electroporation appeared to be safe, provided that delivery was ECG synchronized.\[14\]
REFERENCES


