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Electroporation-based treatments in minimally invasive percutaneous, laparoscopy and endoscopy procedures for treatment of deep-seated tumors

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Abstract. – Electroporation (EP) techniques, used alone (Irreversible Electroporation, IRE) or in combination with anti-cancer drugs (Electrochemotherapy, ECT), have been shown to be effective in the treatment of several types of cancers. The efficacy of ECT and IRE is well demonstrated for the treatment of non-superficial tumor metastases, and it depends on the applied electrical parameters. Particularly, ECT is an effective local therapy that uses electroporation to enhance the cytotoxic effect of bleomycin or cisplatin injected intravenously or intratumorally. Pre-clinical investigations to test alternative anti-cancer drugs, explore new combinations of treatment modalities, and evaluate different sets of pulse protocols for effective tissue electroporation, are ongoing. Further ECT developments include the treatment of deep-seated tumors with percutaneous, laparoscopy, and endoscopy approaches, with the aim of establishing a less invasive approach. ECT is highly effective in the treatment of tumors of any histology, in minimizing the damage of critical normal tissue or organs, and in reducing pain and muscular contractions. This work describes the new technological advances in the field of ECT treatment for deep-seated tumors.

Key Words:
Irreversible electroporation, Electrochemotherapy, Deep-seated tumors, Percutaneous, Laparoscopy, Endoscopy.

Core tip:

The new challenge in the ECT field is the treatment of deep-seated nodules, either percutaneously or with laparoscopic/endoscopic procedures. To this end, new technologically advanced electrodes have been designed to reach and treat tumors that would not be accessible with electrodes already available on the market. Moreover, the electrode design took into account the reduction, as much as possible, of skin burns and both pain and muscle contraction, which are undesired side effects commonly reported in clinical electrochemotherapy studies.

Introduction

The electroporation (EP) was developed around 1970, and its clinical use has spread over the last 10 years, mainly in Europe and the USA. This technique uses electrodes to locally apply electric pulses that determine structural changes in the cell membrane lipid bilayer, creating nanometer-diameter pores. At the cell membrane level, EP occurs when an external electric field above the threshold value of the transmembrane potential (approximately 1.5 V) is applied. An intense electric field, with a local value of at least 400 V/cm[1-3], produces an increase in the cells membrane permeability and conductivity, inducing the formation of pores that allow the entry of non-permeable or scarcely permeable substances that otherwise could not enter the cytoplasm[1-5]. EP can be reversible or irreversible, depending on the cell characteristics (shape, size, cytoskeleton structure, and membrane composition) and the electrical parameters (amplitude and duration of electric pulses, number of pulses, and pulse frequency).

In irreversible electroporation (IRE), a non-thermal treatment that uses short, high-intensity electric pulses, an electric field is induced above the threshold for reversible EP by applying

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to the tissue at least 10 times as many pulses that are normally used for reversible EP[4]. IRE treatment causes the formation of permanent pores and results in cell necrosis; this effect can be successfully exploited to treat both cancers[6-8] and non-cancer pathologies[9-11].

In contrast, reversible EP uses pulses amplitude and duration that allow the formation of pores and also their resealing, preserving the cells viability after the pulse delivery. Reversible EP is largely used in medicine: in combination with an anti-cancer agent (electrochemotherapy, or ECT) or to facilitate the transfer of a DNA plasmid into the cells (electro-gene-transfer, or EGT)[12-18].

Both IRE and ECT have been shown to be effective in the treatment of primary and metastatic tumors[1-4]. The efficacy of ECT and IRE has been demonstrated in metastatic tumors localized in non-superficial organs such as liver metastases[19-25], rectal cancer[26-28], pancreatic cancer[29-33], esophageal tumors[34], and bone metastasis[35]. Unlike hyperthermia-based procedures, such as radiofrequency (RFA), microwave (MWA), high-intensity focused ultrasound (HIFU), and laser therapy[5], which induce coagulative degeneration of the collagen and vascular tissue, IRE and ECT are not thermal methods that can be performed near noble structures (vessels and nerves) without risk of complications. However, when a greater number of electrical pulses is delivered, a more extensive thermal damage[35,36] may be observed around the electrodes. In the case of reversible electroporation, the risk of thermal cells death due to a higher number of pulses and the resulting increase in tissue conductivity near the electrodes' tips can be minimized by adopting an electric pulse protocol in accordance with the European Standard Operating Procedures for Electrochemotherapy (ESOPE)[38-40]. The standard operating procedure protocol consists of 8 pulses with a duration of 100 μs; among these, 4 pulses have a positive wave, and the following 4 pulses have a negative wave. The reference electric field was set at 1000 V/cm, which is widely lower than the one used in IRE treatments.

With careful respect for critical tissue structures, correct electrode positioning and treatment parameters will ensure that the non-thermal benefits of EP are maintained and avoid unnecessary damage to surrounding normal tissue, critical vascular structures, and/or adjacent organs.

New developments in the field of electroporation concern the investigation of new drugs to be used, the combination with other treatment modalities, and the study of new pulse protocols and are aimed at increasing tissue electroporation efficiency and obtaining the so-called "painless electroporation"[41].

Further developments are focused on the ECT treatment of deep-seated tumors with percutaneous, laparoscopy, and endoscopy approaches. A large area of research is dealing with different types of electrodes that can be used *in vitro*, *in vivo*, or in a clinical setting, in open surgery percutaneously or adopting endoscopic approaches[42-46] with different electric pulse types (e.g., sine wave pulses, pulsed electromagnetic field, and high-frequency short bipolar pulses) and electric parameters (amplitude and frequency)[47-51].

The main goal of this study is to provide evidence on the ability of newly developed electrodes in reaching and treating tumors that would not be accessible with electrodes currently available on the market. New electrodes design has also taken into account the reduction, as much as possible, of skin burns and pain and muscle contraction, which are undesired side effects commonly reported in clinical studies on electrochemotherapy.

This work describes the technological advances for ECT treatment of deep-seated tumors.

ECT in Pre-Clinical Studies of Deep-Seated Tumors

ECT treatments of deep-seated nodules, either percutaneously or during laparoscopic/endoscopic procedures, are at their early stages.

Clinical studies in veterinary clinical oncology have demonstrated that ECT treatments may achieve up to 80% long-lasting objective responses in cutaneous and subcutaneous tumors in dogs[52], cats[53], and horses[54]. However, the treatment of not superficial or immediately reachable lesions can be difficult and prolonged. Percutaneous EP is not always possible, and the use of EP with minimally invasive techniques or endoscopic approaches is not yet available.

In vivo effects of reversible electroporation, tested in the normal pancreas in the experimental rabbit model, suggest that ECT may be considered a valid alternative for the local control of non-resectable pancreatic cancer. EP is a safe procedure because it does not affect normal pancreatic parenchyma. Additional *in vitro* studies showed that EP potentiates the bleomycin cytotoxicity in pancreatic tumor cell lines[54,55]. ECT was successfully used to treat nasal cavity tumors in dogs with single-needle electrodes achieving 60% survival rate at 1 year and 30% at 32 months, while 0% was observed for the control group[56]. The safety and efficacy of ECT were also confirmed in 14 dogs with bladder neoplasm showing an improved median survival time[57].

A needle-shaped multipolar probe with telescopic electrodes for percutaneous image-guided IRE and for ECT in solid organs was explored, showing favorable outcomes and feasibility in percutaneous, image-guided, minimally invasive treatment of malignant liver tumors[58].

A novel expandable electrode was used in an experimental brain tumor model in rats. This treatment method was highly effective with complete tumor regression observed in 69% of the treated tumors after one single session, with treatment effects localized to the target area and with a positive toxicity profile; no evident effects on the behavior or morbidity of the rats were observed[59].

An innovative electrode device with optimized geometry, developed for the delivery of antineoplastic drugs and genes in human intracranial tumors, has been shown to improve clinical performance and geometrical tolerance[60]. The first ECT Pre-clinical study in a rat model of brain tumors was published in the early 90's, demonstrating that a double survival time was achieved in the treated rats. A phase I clinical study showed that ECT is safe, effective, and has low toxicity in normal brain tissue[61]. Endoscopic treatment of colorectal cancer, as well as of gastric and esophageal tumors, has been explored with the endoluminal electrode (EndoVe) developed at Cork Cancer Center, University of Cork, Ireland. The EndoVe device has been shown to be safe and effective in spontaneous canine colorectal cancer tumor resolution[62].

Accurate Positioning of the Electrodes

An accurate positioning of the electrodes could improve the ECT treatment, successfully minimizing the risk of an additional intervention[63].

Correct positioning of electrodes, individual long needles, in a variable geometry, requires the support of intraoperative imaging, e.g., X-ray, ultrasound, or computed tomography imaging, to guide the insertion of the electrode. The drawback of incorrect electrode positioning may be insufficient or ineffective EP of the target tissue, as some cells might not be electroporated; as a consequence of the risk of partial responses, local recurrences may occur. For this reason, the treatment needs to be carefully planned with a dedicated software that indicates the exact position of the electrodes and the required EP parameters[64-65]. With this in mind, IGEA S.p.A. (Carpi, Italy) has developed a software[66] that performs the preoperative planning of the electroporation treatment; then it calculates the optimal placement of the electrodes in or around the target area identified (segmented) by the user. The software determines the electric field intensity distribution needed to efficiently electroporate the segmented region, optimizes (i.e., minimizes) the number of electrodes required, and gives a precise indication of the electrode configuration to be used, as well as the voltage and the distance to be set for each couple of electrodes[65].

Another software proposed in the literature[67] is a web-based tool that automatically builds a 3D model of the target tissue from the medical images and then uses this 3D model to optimize the treatment parameters. However, the output file format provided by this tool is not compatible with the commercialized electric pulse generator.

Recently, an additional tool called the EView (https://eview.upf.edu/) has been developed. It is an online platform for 3D electric field simulation in EP-based treatments. EView provides an electric field distribution estimation for arbitrary electrode positions and orientations. It also allows the user to place the electrodes according to potential anatomical landmarks and to visualize the simulated electric field distribution overlaid on the medical image.

The Expandable and Deployable Electrodes

New technologically advanced electrodes have been designed with the main objective of reaching and treating tumors that would not be accessible with the already marketed electrodes. Moreover, the electrode design has also taken into account the reduction, as much as possible, of skin burns and both pain and muscle contraction, which are undesired side effects commonly reported in clinical Electrochemotherapy studies.

Another important aspect to take into account is that, in order to ensure a homogenous distribution of the electric field over the whole volume/surface of the target, the active and conductive parts of the electrodes used must be inserted into the target tissue at the same depth and must be parallel to each other. However, these prerequisites are not easily met when tumor nodules are difficult to access, as is the case of hollow or visceral organs.

For instance, in the field of radiofrequency ablation, a probe that once inserted into the tumor nodule can dislocate or expand single electrodes was developed and showed to cause degenerative coagulation through heat; however, this particular technology is not feasible in ECT treatment, because when the parallelism between the electrodes is lost, there is no guarantee of achieving EP of all tumor cells in the tissue. Indeed, due to the divergence between the adjacent couples of needles, the electric field applied to the conductive part would be extremely in-homogeneous, leaving some areas of the tumor poorly treated.

The setup of a segmentation treatment modality, wherein electrodes with conductive part varying according to the divergence, so that EP of the tumor nodule occurs through successive steps, may be a good solution. The appropriate electric field values needed for each segment will be applied for the EP of all cell membranes.

The family of expandable and deployable electrodes (STINGER electrodes, IGEA S.p.A., Carpi, Italy) makes it possible to apply an effective electric field to all cells to achieve the EP of cell membranes. These electrodes have already been tested on animal models to assess the feasibility and use of the device by performing the treatment in different districts (liver with laparoscopic and open approach, endoscopic using trans-oral and trans-anal via). The electrode configurations allow, in consecutive steps, a gradual increase of the ablated area[68].

The electrode (Figure 1) consists of a shapeable shaft made up of five needles (one central needle and four peripheral needles) running in a multi-lumen and having their heads determine the geometric needle configuration. The needles form the active part of the electrode and, once inserted in the target lesion, allow the electrical pulses to be delivered to the target area (Figure 2). The depth of insertion of the needles is adjustable by means of a control system implemented in the handle. The needles have an insulating polymeric sheath, which limits the conductive part (active part). By means of a graduated scale on the handle itself, it is possible to adjust the insertion depth of the needles up to 4 cm. At different steps, the distances between each needle depend on the initial distance that is determined by the diameter of the shaft and the divergence between peripheral needles and central one.

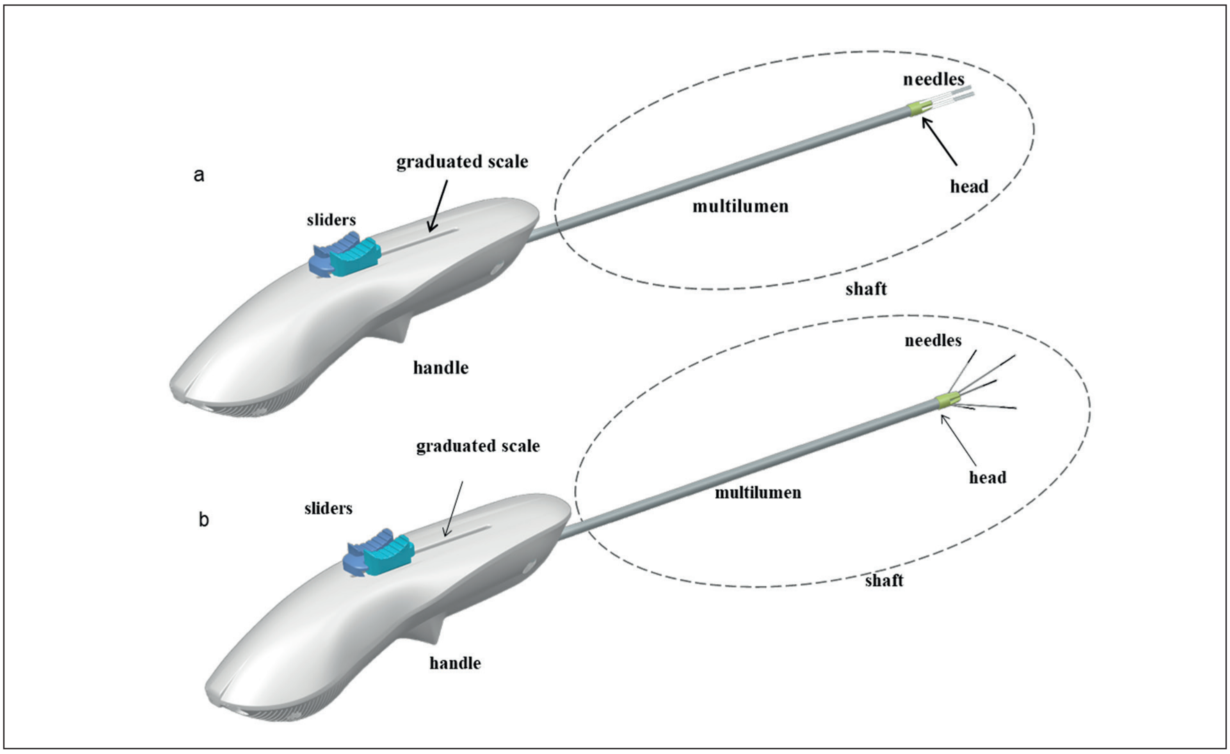

Figure 1. Rendering representation of deployable expandable electrodes with **(a)** zero and **(b)** non-zero divergence (10° of divergence between peripheral needles and electrode shaft axis).

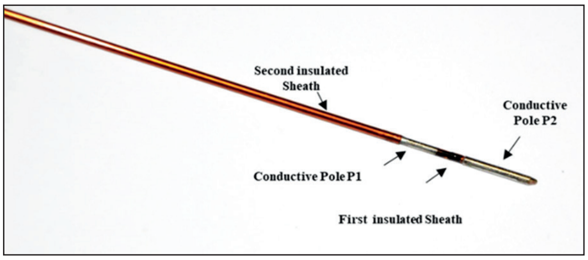

Figure 2. An example of bipolar electrode prototype.

The usability results provided by all the users showed that each electrode model, with and without divergence, tested in laparoscopy/endoscopic surgical approaches[68] and in open surgery, is suitable in terms of usability, mechanical functionality (flexibility, penetrability), visibility of the electrode under radiological guidance, compatibility of the electrode with specific surgical accesses and with the operating room procedure. The handle is satisfactory from an ergonomic point of view, and the flexibility and shape of the invasive element proved to be suitable for the required performance. Safety (no bleeding and/or perforation) and treatment efficacy (adequate ablated volume) was also demonstrated.

Bipolar Electrodes

As already outlined in this article, electroporation-based procedures are influenced by electrode geometry, electric parameters, and local tissue properties. Generally, electroporation studies are conducted with multiple long single needles placed in a variable geometry. The needle insertion should follow an accurate electrode distribution to guarantee the complete coverage of the lesion[69]. This approach is often complained to be time-consuming; moreover, the practice of precisely aiming and accurately aligning a series of multiple electrode needles can be a complicated task in clinical practice[69]. Therefore, new electrode designs, such as a single-insertion bipolar electrode, whereby the anode and cathode are included on a single needle, have been studied[69]. This kind of device reduces the required number of electrodes and simplifies the electrode positioning procedure, saving time, increasing accuracy, and reducing the invasiveness[35-43,69].

Wandel et al[35] investigated a bipolar electrode for IRE treatments in order to optimize and evaluate the effects of the electric condition on the size of treatment zones. Their findings showed that in IRE, the energy generated using bipolar electrodes corresponds to the energy delivered by single-needles systems and that the bipolar needle can increase the ablation volume size.

Bipolar electrodes have also been explored for radiofrequency ablation (RFA) in an endoscopic approach for the treatment of inoperable malignant lesions in biliary structures. This approach prevents stents occlusion, can ablate ingrowth of blocked metal stents, prolong stent patency and also ablate residual adenomatous tissue after endoscopic ampullectomy[40-43].

However, no bipolar electrode had yet been designed for ECT treatment. Igea S.p.A. (Carpi, Italy) explored and studied the optimal design of a bipolar electrode to be connected to a pulse generator[69].

The new electrode represents a compromise between the treatment invasiveness, reducing the diameter of the probe and the electroporated area, which should be at least 10 mm in diameter.

Computer simulation showed that the applied voltage was the main variable influencing the electroporated volume. Furthermore, changing the length of the conductive and insulated poles influenced the distribution of the electric field. The length of the conductive pole determined an increase in the electroporated volume, while the length of the insulated pole inversely affected the size and shape of the electroporated volume: when the length of the insulated pole length decreased, a more regular shape of the electric field was achieved.

The electroporated volume obtained with the electrode of asymmetric geometry was more irregularly shaped than one deriving from the electrode with symmetric geometry. The results of the tests, performed on vegetable samples, showed to be consistent with those of the simulations, and the tests performed on a pre-clinical pig model using the bipolar electrode showed that parenchymal damage of 10 mm was obtained in all experiments with irreversible electroporation protocol.

In conclusion, the invasiveness of the treatment can be reduced by using bipolar electrodes, while ensuring an electroporated volume of about 10 mm in diameter. The bipolar electrode might be particularly suitable for EP-based treatments of deep-seated tumors of limited size. In addition, the bipolar electrode might allow to treat metastases localized to the vertebrae, with a minimally invasive percutaneous approach through the vertebral peduncle.

Advances to Reduce Pain Linked to Electroporation

To enhance the efficacy of EP treatment, the voltage amplitude, duration or number of electric pulses are often increased. However, especially in the treatment of highly conductive deep-seated tumors, there are significant restrictions on parameter settings so that the required current does not exceed the limit set by the pulse generator.

While, the electrical protocol of the Standard Operation Procedure SOPs (a train of eight high-voltage 100-µs long monopolar electric pulses at the repetition frequency of either 1 Hz or 5 kHz)[1,13,39] ensures adequate therapy efficacy, the application of high-voltage monopolar pulses can cause pain and muscle contractions[19].

For this reason, the reduction of both muscle contractions and pain has been investigated over the years. The main improvements were achieved by applying pulses at a higher frequency or by using special electrode designs.

The repetition frequency of the electric pulses is correlated with muscle contraction, which leads to the painful burning sensation and it is often complained by patients[70-72]. Repetition frequency increase, i.e., the reduction in the pulse-to-pulse pause, seems to reduce unpleasant sensations that occur during ECT[51,73-78]. Moreover, many authors reported that microseconds electric pulses at high repetition frequency do not decrease ECT antitumor efficacy[70,73]. More recently, it has been demonstrated that the use of high-frequency irreversible electroporation (H-FIRE)[79-84], namely bursts of short high-frequency bipolar pulses, can further reduce muscle contraction, hence pain, due to the electric pulses. Treatment with H-FIRE pulses, however, might require electric field intensity higher than the standard electric protocol for both ECT and IRE to achieve equivalent treatment efficacy, with the disadvantage of delivering pulses at considerably higher voltage amplitudes.

Hence, applying the pulses in a rapid sequence would be as effective as the use of a larger inter-pulse interval but is better tolerated by the patients[72]. Pulse frequencies above the frequency of tetanic contraction (100 Hz) gradually reduce the number of individual muscle contractions. These results were confirmed by Orlowski and Mir[77], that previously noted that increasing the number, N, of applied pulses leads to a better effect than increasing the pulse duration, T, provided that N×T=const. Reduction of pain due to muscle contractions can be achieved by increasing the pulse repetition frequency above 2 kHz. At this high frequency, patients experienced a single muscle contraction rather than multiple muscle contractions[74].

However, although muscle contraction is related to the pulse frequency, pain sensation also depends on other pulse characteristics such as voltage amplitude, pulse number, pulse duration, and pulse shape[71].

The electrodes used for ECT treatment can also influence the onset of pain. In particular, needles length, diameter, and configuration, i.e., the distance between the needles couple, can be optimized so that the muscle volume crossed by the current is reduced as much as possible. Electrodes with a shorter distance between the couple of needles, on one side, are less painful because they require lower voltages; on the other side, they only allow treatment of small portions of tissue, thus requiring multiple applications to cover the entire lesion.

Other authors have suggested that suitable reversible electroporation and efficient ECT of subcutaneous tumors could be achieved by applying sinusoidal fields, with a significant reduction in muscle contraction[51]. Other authors, instead, have investigated the reduction of morbidity with bipolar/biphasic pulses and observed that altering the pulse polarity reduced the intensity of muscle contractions[84]. Finally, reduction of muscle contraction and morbidity can also be achieved with appropriate electrode design[85,86].

Conclusions

The new challenge for ECT is the treatment of deep-seated tumors, either percutaneously or by laparoscopic/endoscopic procedures. In order to effectively treat the complete tumor and minimize induced damage to critical normal tissues or organs, the following actions may be required:

- The the implementation of pre-treatment planning to achieve an optimal electrode 2D and/or 3D variable configuration that ensures the optimal electric field distribution minimizing the number of electrodes;
- The the use of a minimally invasive surgical approach, such as percutaneous and laparoscopic approaches, using real-time image-guided electrode insertion to allow accurate electrode positioning, according to the treatment plan, for fixed or variable electrode geometries;

- The the development of new minimally invasive electrodes for the treatment of deep-seated intraluminal localized tumors;
- The use of optimized electrical pulses protocol and/or electrode design to reduce pain and muscle contractions.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Authors' Contribution

Roberta Fusco, developed the prototype electrodes. Roberta Fusco and Valeria D'Alessio written the manuscript. Francesco Izzo, Raffaele Palaia and Andrea Belli are the surgeon that performed the pre-clinical testing of the electrodes. Vincenza Granata, Sergio Venanzio Setola and Antonella Petrillo performed radiological images and revised the manuscript.

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