

Electric Field Distribution and Electroporation in Discontinuous Regions Using Vegetal Model: In Vitro and In Silico Study

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Abstract

Electrochemotherapy (ECT) is a recognized electroporation-based technique for treatment of cutaneous and subcutaneous tumors. The treatment is based on cell membrane permeability increase, or electroporation phenomenon, which facilitates the entrance of anti-cancer drugs on cells. Electroporation occurs as function of local electrical field amplitude. Inappropriate electric field distribution can produce tumor recurrence and ECT will fail. This explains the importance of pre-treatment analysis of electrical field distribution and determination of electrical field coverage for electroporation occurrence. The irregularities (e.g., holes and protuberances) may reduce ECT effectiveness as provoke unsuitable electric field. The potato tuber is recognized as an in vitro model of electroporation and provides direct visualization of the affected region. In this study, the impact of protuberance was evaluated using in vitro vegetal model and in silico model. Both analysis demonstrate occurrence of insufficient field for ECT in the protuberance neighborhood.

Keywords

Electroporation • Electrochemotherapy • Electric fields

1 Introduction

Electroporation is a permeabilization increase process which occurs on bilayers membranes after application of intense electric fields. The phenomenon is explained by the phospholipids rearrangement and creation of pores in the cell membrane. These pores allow the passage of ions and molecules into the cell [1].

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Electroporation can be categorized in two levels, depending on the energy applied and cell response. It is called reversible electroporation if after applied fields the pores eventually close. Irreversible electroporation if the applied field is sufficiently intense, which prevents membrane restoration to the original state and cell death results due apoptosis [1].

There are two electroporation-based technologies for cancer treatment. Electrochemotherapy (ECT) introduces chemotherapy drugs (bleomycin) into tumor cells [2]. The cells die in consequence of the drugs action (reversible electroporation). Secondly, irreversible electroporation (IRE) induces cell membrane destruction. This technique exceeds membrane perturbation in a way the pores created cannot reseal, without the need of additional drugs [1]. Both techniques have advantages and disadvantages and they are used successfully in the clinical area [3, 4].

The electroporation protocol used for ECT is eight pulses with 100 μ s duration, 1 Hz repetition rate (i.e. one 100 μ s pulse per second) and amplitude of 100–130 kV/m [5, 6]. This protocol is recommended by European Standard Operating Procedures of Electroporation (ESOPE) created in 2006 [7]. Currently, ECT is used for removal of cutaneous and subcutaneous tumors in European clinics [2, 8] and oral cavities, cutaneous and subcutaneous in Brazilian veterinary clinics [3, 9, 10]. ECT benefits from higher selectivity when compared to other treatment (i.e. surgical removal). Treatment selectivity means the destruction of tumor tissue without destruction of healthy tissue which is important to treat critical and sensitive parts of the body, such as head and neck.

Tumor's disordered growth can reach irregular shapes state. Diffraction of electric field will occur in irregular shapes and electric field distribution may not be simple to predict. For instance, conductivity in boundaries between tumor and air. Regions with insufficient field for electroporation are named blind spots. Blind spots are harmful for ECT, as may produce tumor recurrence. Pretreatment can simulate electrical field distribution to instruct the best procedure for ECT (electrode arrangement and type).

Vegetal models, such as potatoes, could be used to validate the effectiveness of new electrodes sizes and configurations and to assess the impact of pulse parameters (e.g. frequency and number of pulses) [8, 11]. The potato is an electroporation model that allows studying the distribution and intensity of electric fields on tissues in a simplified way. Similar approaches are found in literature [1, 8, 12, 13]. Vegetable models offers advantage as being easily acquired, handled, and reduces animal use in experiments. Besides that, it also has a fast feedback appeal, which are very important for tests involving new configuration for electrodes, non-homogeneous tissues and demonstrations for electroporation devices' users and engineers. On the other hand, in vivo tissue affirmation of ECT occurrence may take weeks, by looking at the tumor reduction and death.

Our aim is to improve the method of potato tuber preparation and emphasize the importance of the vegetal model to show potential risk areas when using ECT. A case of study with cylindrical protuberance was used. The work was conducted in parallel with in vitro and in silico experiments and then compared.

2 Materials and Methods

2.1 In Vitro Study

All the potatoes (*Solanum tuberosum*) were washed and cut until they reached the right shape. The potatoes were acquired at the groceries store the day before the experiment.

The shaping and cutting procedures were made using a tool designed for this experiment, as seen in Fig. 1. This instrument acts as a scissors that does not remove the cylindrical center. The cylindrical shape is made manually using a knife and caliper.

An electroporation device designed on lab for electroporation treatment was used on this study [14]. Eight pulses with duration of 100 μ s, repetition rate of 1 Hz (1 s interval) and amplitude of 100 kV/m was applied on each sample, following ESOPE standard [7] (Fig. 2). The stainless-steel needle electrodes present dimensions of height 150 mm (maximum depth on the tissue), diameter of 0.5 and 3 mm separation. The perforation was performed at the edge of the sample protuberance. Consequently, one of them is fully inside the protuberance and the other is parallel but outside of the protuberance.

A sample control group was also tested along with the group that followed the electroporation procedures. The control group went through the same procedure, including the placement of the electrodes, but without any voltage being applied. The test was run five times (N = 5).

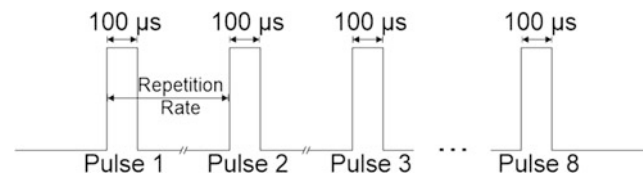
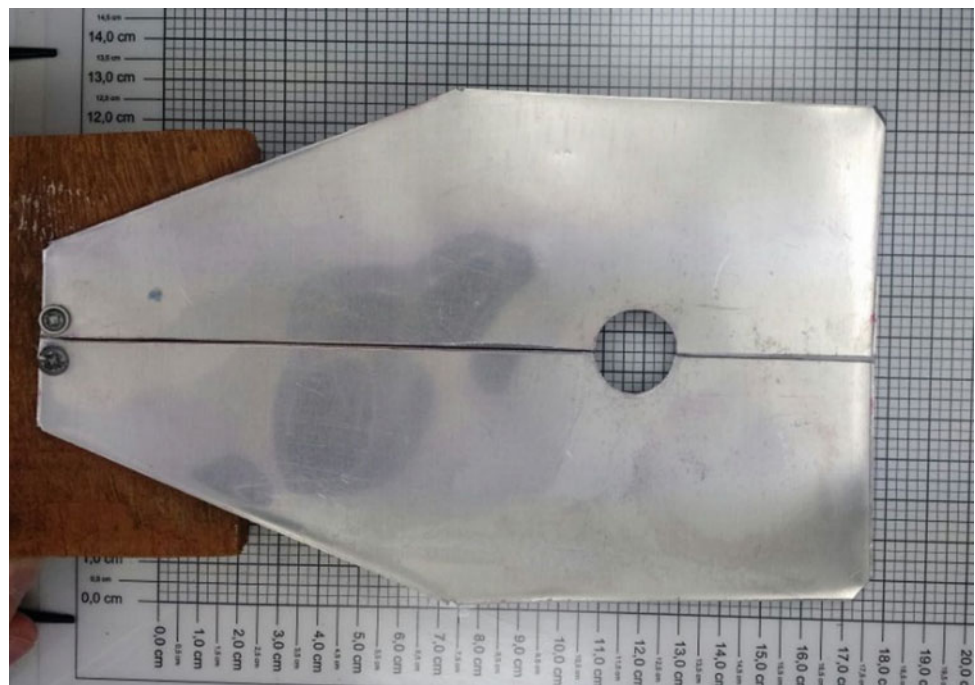


Fig. 2 Pulses applied in the sample

Fig. 1 Tool designed and used to shape the potato samples used in the experiment



The samples were stored in a sealed place with room temperature of 25 °C and in an acid environment (sealed environment with wet paper towel with 2.5 ml of citric acid 4% diluted in deionized water) for a period of 8 h. Acid environment preserves the vegetable tissues (i.e. no natural oxidation).

Electroporation technique overly extracts polyphenol oxidase enzyme which reacts with the oxygen, this reaction leads to a darkening of the vegetable tissue. The marking can be perceived with unassisted vision after 8-h interval. This same mechanism acts when potatoes are cut. Natural oxidation can be reduced if placed on an acid environment [1]. The area affected by electroporation (the darkened areas) where measured with ImageJ.

2.2 In Silico Study

The in vitro geometry according to the in vitro experiment was simulated with three dimensional models using the software COMSOL Multiphysics (COMSOL AB, Sweden). Potato slices were built as geometry of Fig. 3. The two

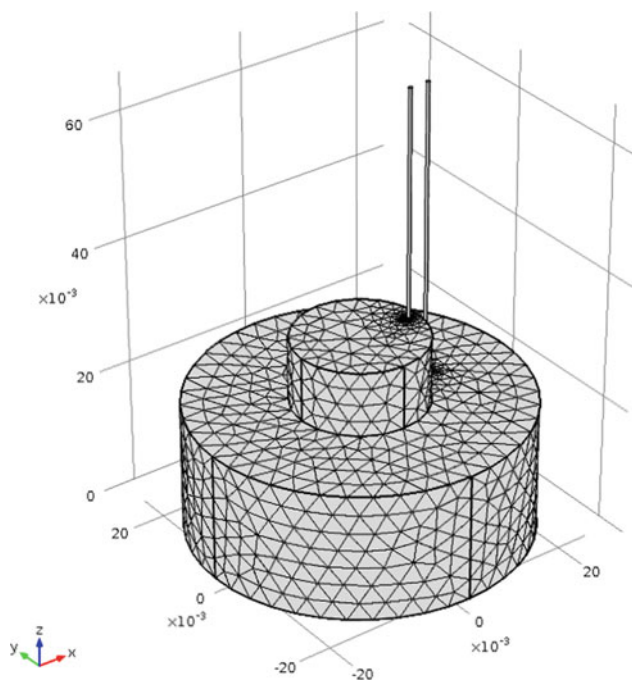


Fig. 3 Geometric model and mesh built in simulation software

needles had 5 cm of height, 150 μm perforation, 0.5 mm as diameter and 3 mm apart. Modeled geometry is presented in Fig. 3. The height of the protuberance is 1 cm, the same as the in vitro experiment. The fine-grained mesh was automatically generated by the software, resulting 41,736 tetrahedral elements in total.

It was considered steady-state regime, and the applied EFs was 130 V/m as ESOPE [5]. The Dirichlet boundary condition (contact between electrodes and tissue), and Neumann (insulating external surfaces) were applied. The tissue was considered homogeneous, and the Laplace Eq. (1) was solved by the finite element method.

$$\nabla \cdot (\sigma \cdot \nabla V) = 0 \quad (1)$$

The parameters of materials needed to compute the simulation are as shown in Table 1.

3 Results

3.1 Lapidate Potato with Protuberance

Processed samples are according to Fig. 4. The cylindrical protuberance represents irregular a tumor.

3.2 In Vitro Results

The in vitro experiment result is shown on Fig. 5. The samples were cut at between needles where the field was applied (side cut). It can be seen on the pictures that the darker area (area that was visibly affected by the electroporation) is concentrated at the area below the protuberance. The darker area average was calculated as 0.33 cm² with standard deviation of ±0.03 cm². In Fig. 5 regions without enough electrical field to electroporation were circled in red circled in situations. This type of situation is known as blind electrical field spot.

3.3 In Silico Results

The in silico results are shown on Fig. 6. The cut plane performed in silico is in the same way as in vitro.

Table 1 Parameters used for simulation

Material	Property	Value
Electrodes (Stainless Steel 405)	Electrical conductivity	1.74 10 ⁹ S/m
Electrodes (Stainless Steel 405)	Relative permittivity	1
Potato tissue	Electrical conductivity	0.03 S/m [1]
Potato tissue	Relative permittivity	1 [1]

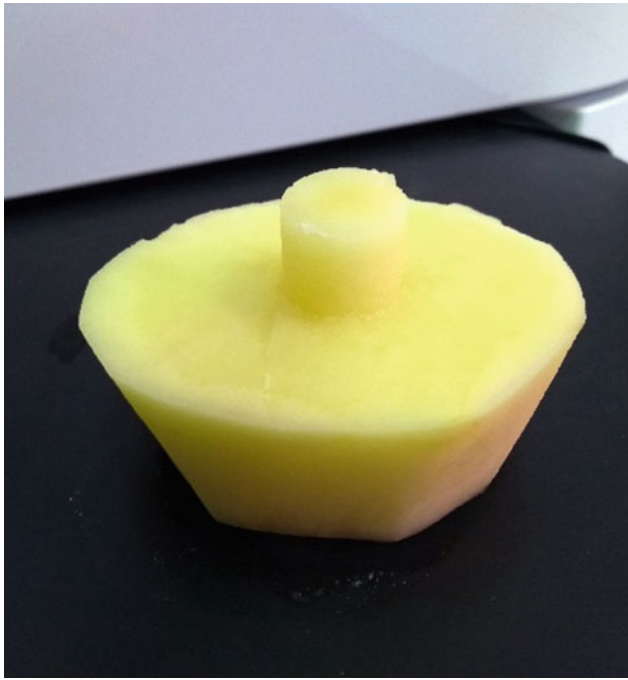
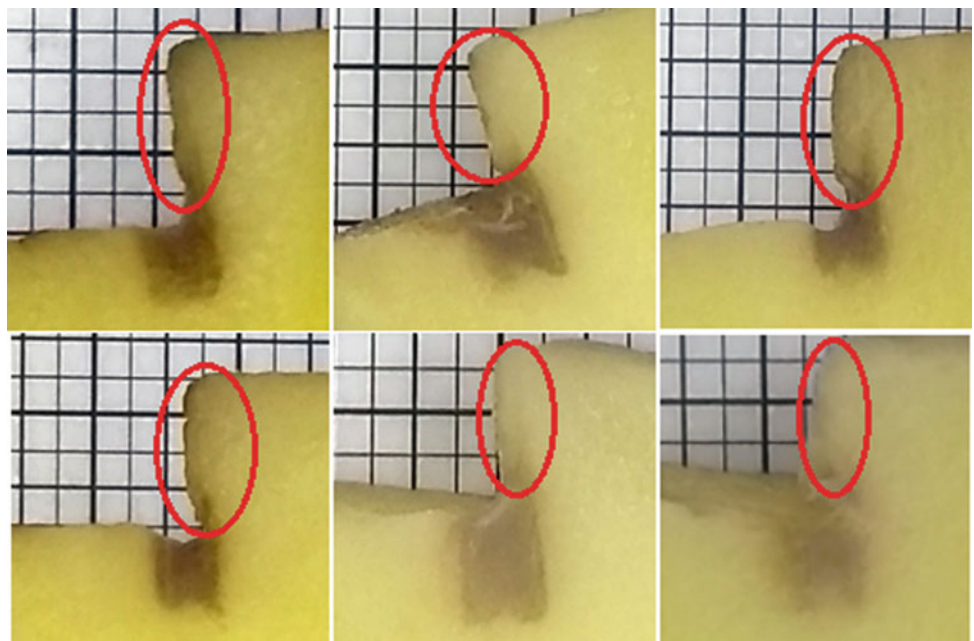


Fig. 4 Samples cut and shaped

The applied electric field was 100 kV/m. The black area occurs when local electric field was higher than 80 kV/m, thus it resulted in irreversible electroporation. On the dark gray area, the electric field was between 20 and 80 kV/m, producing reversible electroporation, which cannot be seen on the *in vitro* experiment samples. The light gray area is where the electric field was below 20 kV/m and it has no significant effects. The software ImageJ was used to measure

Fig. 5 Results showing the lump area using potato model eight hours after the electric field application. In all six images is observed that there is a region of non-electroporation (blind area) shown in red (Color figure online)



the area where the electric field was higher than 20 kV/m and the region where the field was higher than 80 kV/m, results were 0.54 cm² and 0.19 cm², respectively. Similar to *in vitro* (Fig. 5), blind spots are observed in *in silico*.

4 Discussion

The ECT pre-treatment is significant in analysis of electroporation region. The protuberance was analyzed, and a possible treatment failure region was indicated (blind area in Fig. 4 agrees with Fig. 5). The potato model, as exposed in the literature [1, 8, 11–14], has been shown to be an alternative electroporation model to be used in the place of living beings and can provide visual evidence of field distribution.

Both *in vitro* and *in silico* results shown blind spots in the protuberance neighborhood. The electric tissue discontinuous region (protuberance) produces non-uniform electric field distribution. This type of situation is harmful for ECT, as may produce tumor recurrence. The average area found in the *in vitro* results is 40% smaller than the area representing the region where the electric field was higher than 20 kV/m and 73% bigger than the area representing a field higher than 80 kV/m. The difference between measured areas can be explained by the lack of detailed borders in the potato samples, and the regions where the field is weaker and may cause electroporation without noticeable darkening area [15].

Potato models are evaluators of new pulse parameter settings [11]. Based on our studies, it is possible the evaluation of geometric forms with potato model. A case of study with cylindrical protuberance was used. With this type of protuberance, it was possible to show tumor irregular

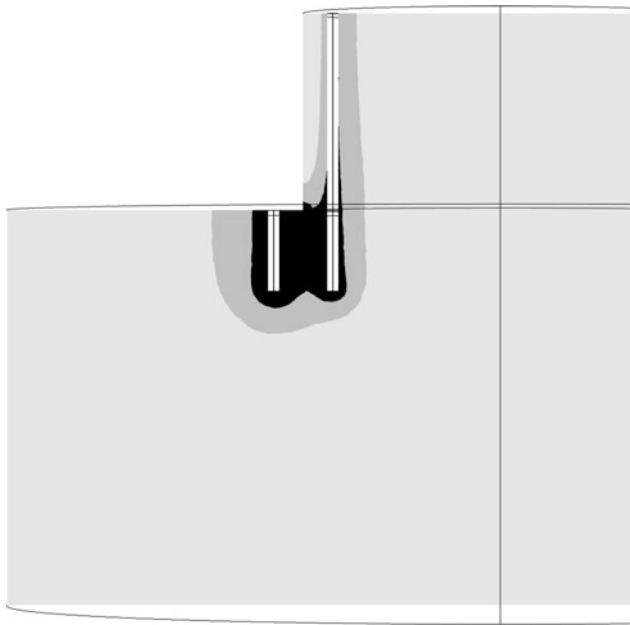


Fig. 6 Result of the in silico study. The black area represents the electric field higher than 80 kV/m. In the dark gray area, the electric field was between 20 and 80 kV/m. The light gray area is where the field was below 20 kV/m

geometry and possible positioning of the electrodes. Future studies can analyze different geometric shapes.

It should be noted that simulations may provide detail and ease of execution of complex geometries, as well as the electroporation conductivity model. However, it is known that the visual appeal of the potato is a fast and inexpensive evidence of success or not of electroporation. In addition, the potato model is interesting in the design of new electrodes, case studies of irregular geometries and feedback for clinicians and engineers. Nevertheless, it is known that the electrical characteristics of the plant tissue are different from an animal tissue [14].

The fine-grained mesh was automatically generated by COMSOL and the experiment converged without apparent noises or discrepancies. The in silico three-dimensional study makes possible the analysis of different types of plane cuts in future works.

5 Conclusion

The in vivo model of potato and in silico studies demonstrated that regions of irregular geometry can impair electric field distribution and cause failure in the ECT treatment. There are indications that the in vitro study can be used as a quick and low cost way of analyzing the effectiveness of ECT and integrity of equipment.

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References

- Ivorra, A., Mir, L.M., Rubinsky, B.: Electric field redistribution due to conductivity changes during tissue electroporation: experiments with a simple vegetal model. *IFMBE Proc.* **25**, 59–62 (2009)
- Pucihar, G., Mir, L., Miklavčič, D.: The effect of pulse repetition frequency on the uptake into electroporabilized cells in vitro with possible applications in electrochemotherapy. *Bioelectrochemistry* **57**, 167–172 (2002)
- Suzuki, D.O.H., Berkenbrock, J.A., de Oliveira, K.D., et al.: Novel application for electrochemotherapy: immersion of nasal cavity in dog. *Artif. Organs* **41**, 767–773 (2017)
- Suzuki, D.O.H., Berkenbrock, J.A., Frederico, M.J.S., et al.: Oral mucosa model for electrochemotherapy treatment of dog mouth cancer: ex vivo, in silico, and in vivo experiments. *Artif. Organs* **42**, 297–304 (2018)
- Marty, M., Sersa, G., Garbay, J.R., et al.: Electrochemotherapy—an easy, highly effective and safe treatment of cutaneous and subcutaneous metastases: results of ESOPE (European Standard Operating Procedures of Electrochemotherapy) study. *Eur. J. Cancer Suppl.* **4**, 3–13 (2006)
- Silve, A., Guimerà Brunet, A., Al-Sakere, B., et al.: Comparison of the effects of the repetition rate between microsecond and nanosecond pulses: electroporabilization-induced electro-desensitization? *Biochim. Biophys. Acta Gen. Subj.* **1840**, 2139–2151 (2014)
- Mir, L.M., Gehl, J., Sersa, G., et al.: Standard operating procedures of the electrochemotherapy: instructions for the use of bleomycin or cisplatin administered either systemically or locally and electric pulses delivered by the Cliniporator™ by means of invasive or non-invasive electrodes. *Eur. J. Cancer Suppl.* **4**, 14–25 (2006)
- Suárez, C., Soba, A., Maglietti, F., et al.: The role of additional pulses in electroporabilization protocols. *PLoS One* **9**, e113413 (2014)
- Calvet, C.Y., Famin, D., André, F.M., Mir, L.M.: Electrochemotherapy with bleomycin induces hallmarks of immunogenic cell death in murine colon cancer cells. *Oncoimmunology* **3**, e28131 (2014)
- Suzuki, D.O.H., Marques, C.M.G., Rangel, M.M.M.: Conductive gel increases the small tumor treatment with electrochemotherapy using needle electrodes. *Artif. Organs* **40**, 705–711 (2016)
- Bhonsle, S.P., Arena, C.B., Sweeney, D.C., Davalos, R.V.: Mitigation of impedance changes due to electroporation therapy using bursts of high-frequency bipolar pulses. *Biomed. Eng. Online* **14**, S3 (2015)
- Castellví, Q., Banús, J., Ivorra, A.: 3D assessment of irreversible electroporation treatments in vegetal models. In: *IFMBE Proceedings*, pp. 294–297 (2016)
- Hjouj, M., Rubinsky, B.: Magnetic resonance imaging characteristics of nonthermal irreversible electroporation in vegetable tissue. *J. Membr. Biol.* **236**, 137–146 (2010)
- Berkenbrock, J., Pintarelli, G., Antônio, A., Suzuki, D.: In Vitro simulation of electroporation using potato model. In: *CMBES Proceedings* (2018)
- Winnipeg, C., May, M.B.: Electrochemotherapy I, Electroporation I 2017 CMBEC40 Conference, Winnipeg MB, 23–26 May 2017