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Computer simulation of commercial conductive gels and their application to increase the safety of electrochemotherapy treatment

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ABSTRACT

Electrochemotherapy (ECT) exploits the phenomenon of electroporation, which is the increase of cell permeability through the application of an electrical field. This technique is applied in medical centers in Europe and in veterinary clinics in Europe, Brazil, and Argentina. ECT treatment requires a minimum electric field and anti-cancer drugs (*e.g.*, bleomycin). Irregularly shaped tumors may induce ECT treatment failure because of irregular electric field distribution. Conductive gels have been suggested as a means to increase the homogeneity of the electrical field distribution. The aim of this work was to evaluate if commercial conductive gels could increase the safety of ECT. A veterinary case study of ECT in a dog provided the tumor dimensions for the numerical model. Electrode displacement and commercial conductive gels were simulated to determine if they improved ECT treatments. We conclude that a commercial gel having a conductivity of 0.2 S/m when used in combination with effective treatment planning may improve the outcome of electrochemotherapy procedures.

1. Introduction

Melanocytic tumors represent 4–6% of dog skin tumors [1]. Cutaneous melanoma has a higher occurrence among 7–14-year-old dogs [1,2]. The majority of malignant melanoma cases in dogs occur in the oral cavity and the mucocutaneous junction of the lips. For malignant melanoma removal, the traditional treatment involves a wide surgical incision; however, the prognosis for malignant melanoma is poor because of the high incidence of metastasis [2]. To reduce recurrence and metastasis, incision margins are used when removing the primary tumor and tumor cells already spread to surrounding tissues. Excision margins for cutaneous melanoma range from 10 to 50 mm; however, there is little agreement about safe incision margins [3].

Electrochemotherapy (ECT) is an innovative local cancer treatment in which electric fields (EF) are applied with chemotherapy drugs (*e.g.*, bleomycin and cisplatin) for the removal of cutaneous and subcutaneous tumors [4,5]. ECT procedures usually comply with the European Standard Operating Procedures of Electrochemotherapy (ESOPE) [13], which were published in 2006 and updated in 2018 [14]. In European clinics, ECT has been used to effectively treat cutaneous melanoma [6,7]. ECT is also used in veterinary clinics in Europe, Brazil, and Argentina to treat cutaneous, oral cavity, nasal duct, and colorectal tumors [8–12]. Although pain and muscle contractions have been observed in patients undergoing ECT, the treatment has several advantages over other cancer treatments, including improved tumor-selectivity, increased immune system response, and antihemorrhagic effects; its main benefit, however, is that it avoids the need to remove an extended tissue area (*i.e.*, safety margin) in critical parts of the body, such as the head and neck [15]. For head and neck tumors, the removal of extensive tissue can cause disfigurement, reduce function, and limit the treatment of the tumor. Using ECT, healthy tissue is preserved, and tumor cells are eliminated due to ECT selectivity. Anticancer agents can be applied intravenously, and the safety margin around the tumor can be guaranteed. Thus, ECT can do more than provide good cosmetic results; it can also preserve the safety margin that is critical to the prognosis of the disease [16].

The basis of ECT is electroporation (EP), the cellular permeability-increasing phenomenon that occurs when cells are exposed to EF greater than tens of kV/m at a duration of nanoseconds to seconds. Processes such as molecule uptake indicate that permeability increases due to pores formed in the cell membrane [17–19]. EP analysis is mainly done indirectly through

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investigation of impedance and/or conductivity sample changes, computer simulations, and fluorescent marker intake during the procedure [4,5,20]. The electrical conductivity indicates the ability of certain material to conduct an electric current. Determining the EF distribution in a medium depends directly on knowledge of the electrical conductivity and current distribution.

The success of the EP technique and consequently of ECT treatment is a function of EF distribution [13,21–23]. Physically non-homogeneous and geometrically irregular mediums may produce diffraction of EF distribution in boundaries (*e.g.*, tumors and air). EF distribution computer simulations are used to predict ECT success [9,10,24–26]. These simulations are part of ECT pre-treatment or treatment planning [27–29]. Adequate gel conductivity can improve the distribution of EF in boundaries and reduce the contact impedance between the electrodes and the skin [9,24].

In previous studies, gel applications with ECT have demonstrated uniform EF distribution with plate [24] and needle electrodes [9]. These fixed distance electrodes are applied to small tumors (diameter less than 5 mm). The gel solves the limited distance between electrodes and reduces areas of limited electrical contact. In this work, we demonstrate that commercial gels could improve the efficiency and safety of ECT with large tumors (diameter greater than 5 mm). Treatments with 100 kV/m and lower EF applications were applied with different electric conductivity gels and an *in silico* model to evaluate ECT efficiency. Here, an *in silico* model was validated using a veterinary case study of melanoma in a dog. Miklavcic's electroporation tissue model [25] was implemented. The treatment effectiveness (occurrence of electroporation) was verified *in silico* with and without two different electrode displacements and with and without the addition of gel

2. Materials and methods

The conductivity of four types of commercially available conductive gels was measured. The boundary conditions that were input into the numerical model (tissue geometry and tumor dimensions) were obtained from the canine ECT case study. Thus, *in silico* experiments with EF and with and without electrode displacement were executed. The experiments used two types of typical electrode displacement (*x* and *y* directions) and were conducted with and without conductive gel.

2.1. Experimental conductivity analysis

The conductivity of four conductive gels was examined at a driving voltage of 1 V and frequencies from 1, 5, and 10 kHz using an impedance analyzer HP4194A (HP Agilent, Inc., California, USA). The test fixture HP16024E (HP Agilent, Inc., USA) was used to couple an additional developed accessory. All measurements were made at 25 °C. In addition, three individual measurements were repeated for each value.

A measurement device was developed to be coupled with the impedance analyzer fixture. A cylindrical support with a diameter and length of 15 mm was produced. In the cylinder bases, gold electrodes were used. The gels were placed in the main cylindrical compartment, and measurements with the impedance analyzer were performed. The electrodes were cleaned thoroughly after each measurement. The accessory in Fig. 1 was developed to improve the accuracy of the electrical measurements. Electrical conductivity (σ) depends on material length (l), the electrodes–material contact area (A), and electrical resistance (R), as in Eq. (1) [30].

$$\sigma = \frac{l}{A \cdot R} \tag{1}$$

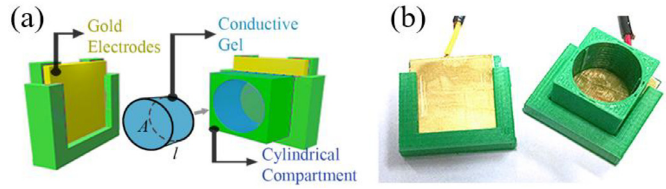

Fig. 1. The developed cylindrical fixture with a diameter and length of 15 mm. Gold electrodes were used. (a) Area (A) and length (l) of the schematic cylinder used for gel conductivity calculation (Eq. (1)). (b) The geometry was designed in 3D software. This accessory improves the accuracy of electrical measurements.

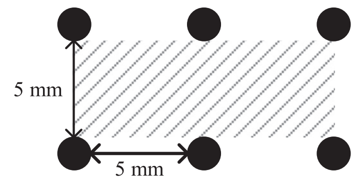

Fig. 2. Three needle-type electrode pairs. The hashed area represents the tissue content to be treated with ECT. The needle-type electrodes permit electric fields to reach deep tissues beyond the visible tumor volume. These safety margins minimize potential microscopic invasion.

2.2. *In vivo electrochemotherapy*

A 9-year-old mixed breed dog, 23 kg, was diagnosed with melanoma cancer in the mucocutaneous region. The nodule was pink, single, exophytic, 20 mm in diameter, ulcerated, and alopecic. The dog was submitted to a general anesthetic. The drug used for ECT was bleomycin IV (3000 IU/mL). Seven minutes after the drug injection, the ECT protocol was applied in a single session using the Vet CP 125® electroporator (Vetcancer, São Paulo, SP, Brazil). The protocol consisted of eight bipolar pulses of 100 µs duration, 70 kV/m, and a frequency of 5 kHz. Stainless steel needle-type electrodes with three pairs of needles were used without the application of conductive gel, as shown in Fig. 2. The patient was clinically monitored for 120 days, without recurrence.

2.3. Numerical modeling

Simulations were executed to solve the continuity equation under the assumption of a steady-state situation (Eq. (2)). The tetrahedral elements mesh was assembled in the software COMSOL Multiphysics® version 4.4 (COMSOL, Inc., Sweden). A Windows 10 (64-bits) operating system (Microsoft Corporation, WA, USA) and Core i7 processor (Intel Corporation, CA, USA) were used [30].

$$\nabla \cdot J = -\nabla \cdot (\sigma \nabla \cdot V) = 0 \tag{2}$$

where $\boldsymbol{J}$ is the current density (A/m²), σ is the tissue electrical conductivity (S/m), and V is the electric potential (V). In Eq. (3), V is used to compute the electric field $\boldsymbol{E}$. In Eq. (4) (Ohm's law), $\boldsymbol{E}$ is used to compute the current density $\boldsymbol{J}$ [30].

$$E = -\nabla \cdot V \tag{3}$$

$$J = \sigma E \tag{4}$$

The boundary conditions that were input into the numerical model were all insulated on the external surfaces (Neumann's boundary condition). The contact between the electrodes and tissue was modeled as Dirichlet's boundary condition.

In addition, it is necessary to consider the phenomenon of electroporation, in other words, the change of electrical conductivity in biological tissues as a function of the local applied electric field (E). A suitable model should be used, such as the model of Sel et al. [25], as expressed in Eq. (5). This model consists of a sequence of

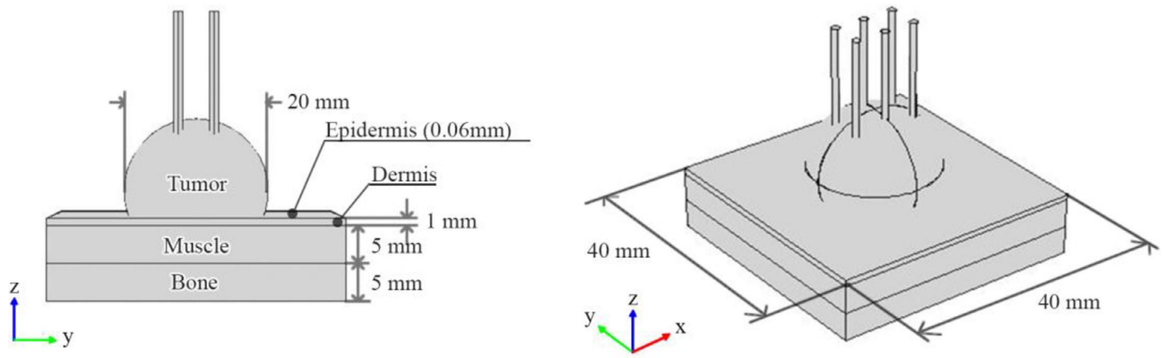

Fig. 3. Three-dimensional geometry used to simulate the case study.

Table 1
Materials used for *in silico* simulation and their electrical conductivity values. Electric field thresholds for irreversible (E_{irrev}) and reversible electroporation (E_{rev}). Values of the initial (σ_0) and maximum (σ_{Max}) electrical conductivity of tissues.

Material	Electrical conductivity [S/m]		E_{rev} [kV/m]	E_{irrev} [kV/m]
	Initial electrical conductivity (σ_0)	Maximum electrical conductivity (σ_{Max})		
Electrodes[a]	1.74×10^9		–	–
Tumor[b]	0.300	0.750	40	80
Epidermis[b]	0.008	0.800	40	120
Dermis[b]	0.250	1.000	30	120
Muscle[b]	0.135	0.340	20	80
Bone[c]	0.01		–	–

[a] Data from the COMSOL library (Stainless Steel 405).
[b] Data from Corovic et al. [31].
[c] Data from Gabriel et al. [32].

static models, which describe the approximate E distribution under tissue electropermeabilization. The model is based on sigmoid dependency between conductivity and E intensity. This model has been studied by Suzuki et al. in different circumstances of ECT treatment [9,10,26].

$$\sigma(E) = \sigma_0 + \frac{\sigma_{Max} - \sigma_0}{1 + D \cdot e^{-\left(\frac{E-A}{B}\right)}} \quad (5)$$

where,

$$A = \frac{E_{irrev} + E_{rev}}{2}, B = \frac{E_{irrev} - E_{rev}}{C}, C = 8 \text{ and } D = 10$$

The tissue geometry and tumor dimensions were obtained from the canine ECT case study. Three-dimensional geometry with all geometry dimensions is shown in Fig. 3. The mesh consisted of 387,213 to 426,611 tetrahedral elements (depending on the displacement). Electrode dimensions are presented in Fig. 2. The necessary parameters for computing the electrical conductivity of tissues (Eq. (5)) are initial and maximum electrical conductivities, as well as EF amplitudes for irreversible (E_{irrev}) and reversible electroporation (E_{rev}). These parameters are shown in Table 1.

The ECT applications of EF are made repeatedly in diverse locations in order to exceed the boundaries of the tumors and keep the safety margin. Because of this, physicians and veterinarians face situations in which the electrodes may be displaced out of the tumor volume [26]. Here, electrode displacement in two directions (x and y-axes) was studied. The geometries are shown in Fig. 4(a) and (b). For the gel study, a rectangular shape 15 mm in height covering the entire tissue surface was added. This represents the conductive gel, as shown in Fig. 4(c). When the EF between the electrodes (*i.e.*, area shown hashed in Fig. 2) is suitable for EP, ECT

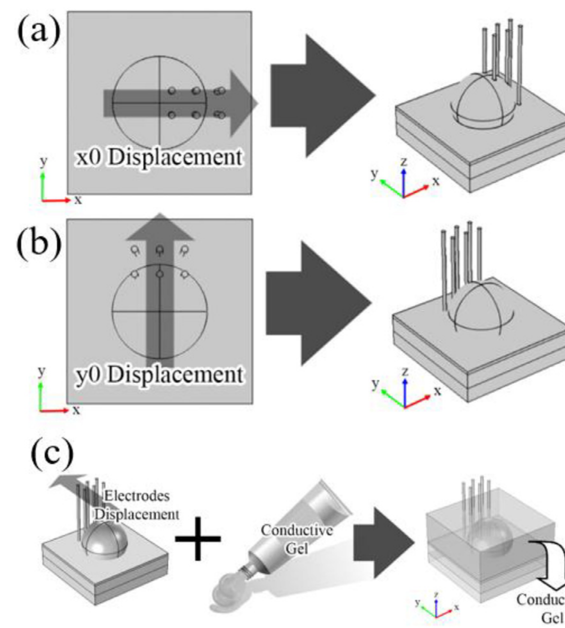

Fig. 4. Electrode displacement geometries at x and y-axes 10 mm. (a) x0 displacement and (b) y0 displacement. (c) Conductive gel addition. The electric fields may cover the entire tumor volume and safety margin.

success is likely. Therefore, this was used as the definition of ECT success.

3. Results

3.1. Veterinary case of study

The same 9-year-old dog was admitted for an ECT clinical follow-up, and records were made through images of the different tumor conditions over time, as shown in Fig. 5. The ECT protocol constituted eight bipolar pulses of 100 µs in duration, at 70 kV/m, and at a frequency of 5 kHz. Needle-type electrodes with three pairs of needles were used, as shown in Fig. 2. A histological analysis after 120 days did not detect tumor recurrence. In conclusion, the ECT treatment was successful.

3.2. Evaluation of commercial gels

The electrical conductivity of the four conductive gels was measured; the data are presented in Table 2. Lower and upper conductivity gel limits were from 0.1 to 0.2 S/m. These conductivity values were used in the computational simulation with gel.

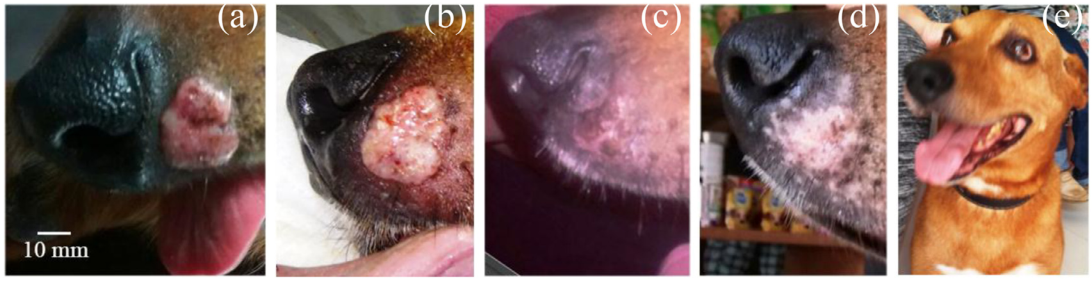

Fig. 5. (a) Before ECT, (b) On the day after ECT, (c) 7 days, (d) 15 days and (e) 120 days after ECT without tumor recurrence.

Table 2
The conductive gels used in this study and their respective conductivities measured using an impedance analyzer "Agilent 4294A".

Commercial gel	Electrical conductivity (σ) [S/m]		
	1 kHz	5 kHz	10 kHz
Gelytec, produced by Caithec	0.154 ± 0.004	0.150 ± 0.020	0.150 ± 0.020
Carbogel ECG, produced by Carbogel	0.128 ± 0.001	0.129 ± 0.010	0.130 ± 0.001
Ultra Gel – Ultrasound Gel, produced by Multigel	0.190 ± 0.010	0.197 ± 0.001	0.197 ± 0.001
Carbogel ULT, produced by Carbogel	0.103 ± 0.003	0.104 ± 0.003	0.104 ± 0.003

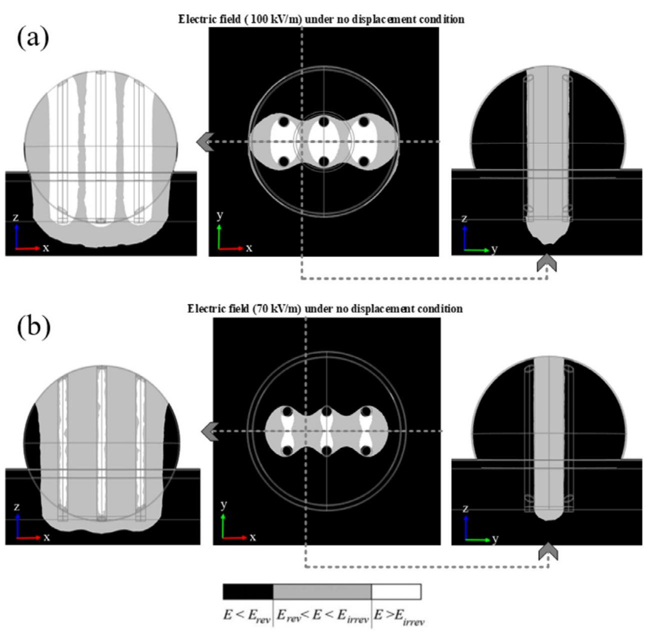

Fig. 6. No displacement situations. In all results, black indicates no electroporation (EF below tumor E_{rev}), white indicates irreversible electroporation (EF higher than tumor E_{irrev}), and gray indicates reversible electroporation (EF between tumor E_{rev} and E_{irrev}). Arrows indicate the direction of plane cuts. (a) 100 kV/m was applied. Most of the area between electrodes had EF above 80 kV/m (white area). (b) 70 kV/m was applied. This is the minimum EF capable of electroporating all the tumor content between needles.

3.3. In silico *evaluation*

3.3.1. Without gel application

The success of ECT treatment observed *in vivo* in a veterinary case study indicates that EP occurred in the tumor area. During the ECT treatment, a series of applications was made in which the electrodes were displaced in several ways. The 100 kV/m protocol (500 V) with no displacement was simulated. The results shown in Fig. 6(a) indicate that ECT was successful. However, as can be seen, the area between electrodes had mostly sufficient EF for irreversible EP (white area). Thus, the minimum voltage value for no displacement was simulated. It was possible to achieve ECT success with an EF as low as 70 kV/m (350 V is the minimum voltage required to electroporate the area between the inner walls of the electrodes). The simulation results are shown in Fig. 6(b). In all *in silico* results, black indicates that the EF is below 40 kV/m (no EP case, or below the tumor's reversible EP threshold E_{rev}), white indicates that the EF is higher than 80 kV/m (irreversible EP case, or higher than the tumor's irreversible EP threshold E_{irrev}), and gray indicates reversible EP (EF between E_{rev} and E_{irrev}). Arrows in the cutting plane (B) indicate the direction of the two plane slices (A and C).

It was possible to achieve ECT success in both 100 kV/m and 70 kV/m protocols if the electrodes were displaced by 10 mm x0 out of the tumor volume. However, using a displacement of 10 mm y0, a blind EF spot was observed. An insufficient EF area could result in ECT treatment failure. Electrode displacement results are shown in Fig. 7.

3.3.2. With gel application

As shown in Fig. 7, y0 displacement may cause treatment failure. Thus, caution is needed when using this type of displacement. Accordingly, conductive gel was added to *in silico* simulations to fill air gaps, thus enhancing ECT safety. Two values of gel conductivity were used: 0.2 S/m and 0.1 S/m. It was possible to achieve ECT success using 100 kV/m, y0 displacement, and 0.2 S/m conductive gel, as shown in Fig. 8(a). In this configuration, the minimum EF amplitude for ECT was 80 kV/m (or 400 V), as shown in Fig. 8(b). The use of 0.1 S/m gel improved the EF distribution; however, this gel could not drive successful ECT with 100 kV/m and y0 displacement. This arrangement produced blind EF spots, as shown in Fig. 8(c).

3.3.3. Electric currents at electrodes

Electric currents to ensure the voltage applied between electrodes for the 100 and 70 kV/m situations with and without gel and displacements are shown in Table 3. The superscript 'X' denotes if the parameters were not sufficient to ensure EP between the interior of the electrodes, in other words, situations where the ECT treatment may fail.

4. Discussion

When using ECT, physicians need to cover the entire tumor volume with adequate EF. Accordingly, knowledge of electrode dis-

Table 3
Electrical currents needed to cover all the situations studied in this work. 'X' denotes situations where the ECT treatment may fail.

	EF applied and displacement					
	70 kV/m none	70 kV/m x0	70 kV/m y0	100 kV/m none	100 kV/m x0	100 kV/m y0
Without conductive gel	10.7 A	7.4 A	2.1 A^X	19.0 A	13.1 A	4.0 A^X
With conductive gel 0.1 S/m	11.2 A	8.4 A	4.1 A^X	19.7 A	14.7 A	6.9 A^X
With conductive gel 0.2 S/m	11.7 A	9.3 A	6.0 A^X	20.4 A	15.8 A	9.7 A

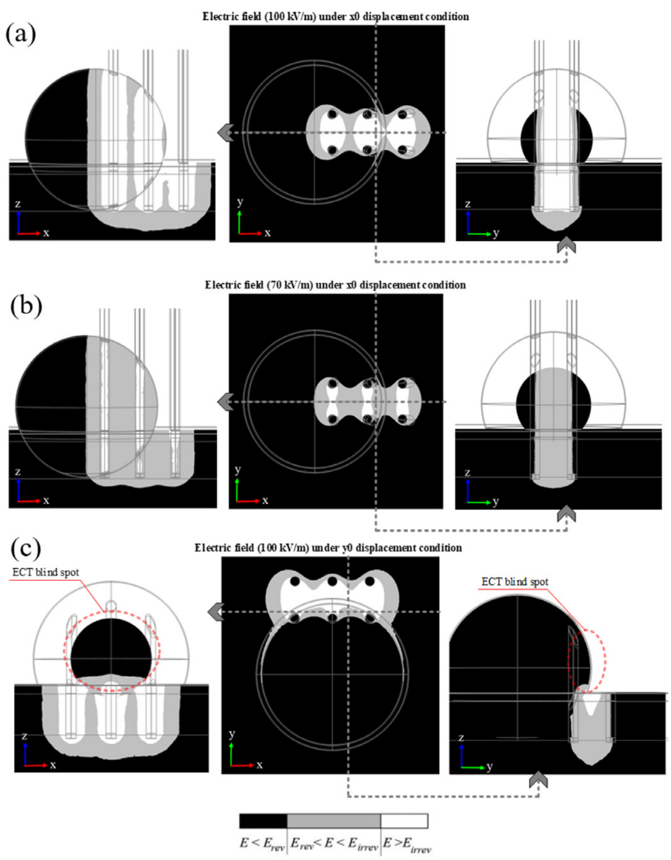

Fig. 7. The figure shows the results for 10 mm displacements. (a) 100 kV/m with x0 displacement. (b) 70 kV/m with x0 displacement. (c) 100 kV/m with y0 displacement. Hence, both (a) and (b) are suitable for ECT. In (c), a blind EF spot (red dotted circles) was observed. This indicated a possible ECT failure. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

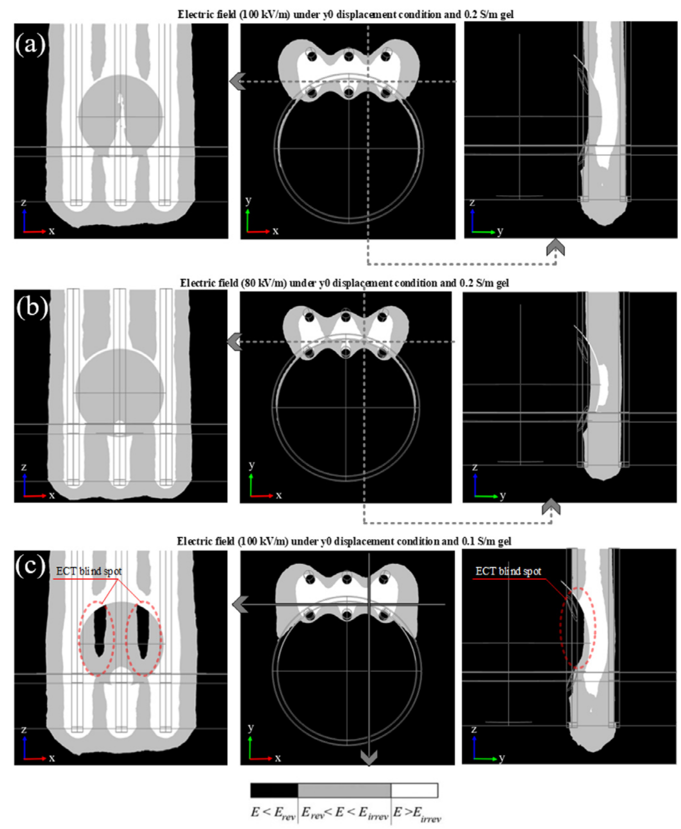

Fig. 8. Simulations using conductive gels and 10 mm y0 displacement. (a) 100 kV/m with 0.2 S/m gel. (b) 80 kV/m with 0.2 S/m gel (this is the minimum EF to perform ECT in this setup. (c) 100 kV/m with 0.1 S/m gel. In (c), a blind EF spot (red dotted circles) was observed. The 0.2 S/m gel improved EF distribution, while 0.1 S/m gel produced possible treatment failure. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

placement is essential. This work addresses two situations of displacement that may occur in clinical treatment and considers if commercial conductive gels may help improve the safety of ECT treatment.

ECT can be successful if there are anti-cancer drugs available in the tumor tissue and the local EF distribution is over 40 kV/m [21]. An *in vivo* veterinary case study provided an *in silico* model with the geometry of a dog's tissue and tumor dimensions. The relationship between electrode displacements and the conductivity of commercial gels was simulated numerically. To avoid animal experimentation, in keeping with the 3R concept for animal research, it is recommended that gel and displacement techniques be tested *in silico* prior to *in vivo* animal tests [33]. In situations where ECT fails and areas are not covered with EF, the neoplasm will proliferate, and tumor cells can metastasize [21]. In the y0 displaced without conductive gel (Fig. 7(c)), some tumor areas were not electroporated without gel (blind spots). When commercial conductive gels of 0.2 S/m were applied over the tumor, EF was sufficient to electroporate the tumor (Fig. 8(a) and (b)).

When comparing the two displacements without gel, the y0 displacement failed for ECT even with 100 kV/m. Consequently, y0 displacement (Fig 7(c)) should be avoided. The x0 displacement may be more successful in covering protruding and irregular geometries (Fig 7(a) and (b)).

The *in silico* experiment allowed lower EF amplitude than the ESOPE procedure (100 kV/m) [23]. Avoiding an excessive amplitude of EF may be critical as it can cause irreversible EP. Based on our study, an EF drop as low as 80 kV/m may be enough for ECT treatment. The protocol of 70 kV/m was not satisfactory for the use of 0.2 S/m gel and y0 displacement (data not presented). This situation illustrates why treatment planning is significant in ECT. Moreover, the ECT electrodes suggested by ESOPE are type III (hexagonal electrodes), and the equipment used a different electrode configuration, as seen in Fig. 2.

Conductive gels are currently used in ECT to improve contact between electrodes and cutaneous tumors [17]. However, conductive gels can also have a supplementary application for ECT. The use of gels increases the safety margins of ECT and provides uniform EF distribution on the tumor [22]. The optimum electrical conductivity value is 0.5 S/m [9,24]. However, commercial conductive gels do not present the ideal conductivity value (Table 2).

The use of conductive gel increases electrical current consumption by up to 10% when the highest current application without gel is compared with the highest current application with gel. Therefore, the use of a gel should not be problematic for the equipment. Even so, the current measurements might be used to predict situations in which the treatment fails. When electrical currents are lower than 7 A, the equipment triggers an alarm signifying failure.

Electrical currents and smaller EF amplitudes can reduce current densities near the electrodes, reducing the possibility of thermal effects. These features trigger the immune response and reduce the likelihood of scar formation and tissue necrosis [34–36].

Full tumor removal was achieved through ECT in the case study, with preservation of the healthy tissue. The 100 kV/m applied EF demonstrated effective ECT in both the *in vivo* experiment and the *in silico* model. However, the *in silico* study showed that y0 displacement left an area of the tissue unelectroporated (Fig. 7(c)). During ECT, physicians and veterinarians may face situations in which the electrodes have to be displaced from the tumor volume, and they may therefore perform multiple non-quantized displacements. Gels may be a solution to avoid the possibility of failure and to improve the safety of electrochemotherapy.

The surgery margin recommended is 10–50 mm for most lesions [3]. In the presented case, traditional surgery was a possibility but would have resulted in mutilation of the upper lip and snout. ECT was a viable solution as it does not require mandatory tissue removal [26].

The ECT numerical model previews irreversible areas (Figs. 6 and 7), but the *in vivo* case presented no evidence of irreversible areas (Fig. 5). The high EF values predicted by the numerical model may occur in the tissue, However, high EF occurs with a few short pulses (eight pulses of 100 µs). Irreversible electroporation treatment techniques use 10 times more pulses [37,38].

5. Conclusion

ECT treatment may fail because the local EF is not sufficient in some areas of the tissue to electroporate malignant cells. Conductive gels of 0.2 S/m avoid blind spots when EF are applied and are already used to increase the skin-electrode' contact. Accordingly, they are recommended for use during the ECT procedure. We recommend that to avoid infection when using needles electrodes, the gels should be sterile because of the perforation and contact with the skin. Additionally, the ESOPE protocol recommends the use of 100 kV/m [23]. However, it is possible to use protocols as low as 70 kV/m, as studied with *in silico* simulations and a veterinary case study.

Human and animal rights

This work did not influence the clinical procedure of electrochemotherapy (ECT) treatment. This work only used the data obtained from the clinical treatment: tumor dimensions and ECT treatment protocol (amplitude and electrode dimensions). The study, which had no bearing on the care of the animal undergoing treatment, was carried out in accordance with the recommendations of the Ethics Committee for the Use of Animals of Federal University of Santa Catarina (no protocol was required).

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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