Conductive Gel Increases the Small Tumor Treatment with Electrochemotherapy using Needle Electrodes

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Abstract:
The combination of chemotherapy drugs and high electric field treatment in local cancer is named electrochemotherapy. European standard operation procedure of electrochemotherapy (ESOPE) provides guidelines for treatment of cutaneous and subcutaneous tumors. The electrochemotherapy of numerous tumors varying in sizes is more convenient using needle electrodes. However, ESOPE recommends that needle electrodes are applied to deeper tumors. The application of needle electrodes to treatment of superficial small tumors seems to be practical in electrochemotherapy. Plate electrodes and gel improve the electrochemotherapy efficacy. This technique provides electric field homogeneity in irregularly shape tissue structure (bulk tumours). We propose an investigation of needle electrode and gel in electrochemotherapy of superficial tumours. *In vivo* experiment with SCC (squamous-cell carcinoma) spontaneous nodules in dog was used to validate the mathematical tissue model. The numerical model considers the tissue conductivity dependent on local electric field. Our studies demonstrated that conductive gel is important for effective treatment of superficial tumour with needle electrodes. The needle electrodes and gel presented reduction of medium current, increase the tumour free margin and improve the practical application in relation to plate electrode.

Key words: Electrochemotherapy, Conductive gel, Tumor, Squamous-cell carcinoma, SCC.

Introduction

Electrochemotherapy is a local cancer treatment with the combination of high local electric field and chemotherapic drugs [1], [2]. This treatment reduces the damage on adjacent healthy tissue that occurs during surgical resection. The electrochemotherapy is based on the electroporation or electropermeabilization of tissues. The electroporation increases the permeabilization of the cell membrane to substances, like drugs and DNA, into the cells. Consequently, this phenomenon increases the electric conductivity of biological tissues [3]–[6]. The planning of electrochemotherapy is an important step to avoid clinical mistakes. Tumours regrew in the areas where the local electric field is not enough. Numerical models of the treatment need to provide the electric field dependence of tissues. It reduces the prediction error from 30% to 3% [7].

The European Standard Operation Procedure for Electrochemotherapy (ESOPE) defined and validated the procedures for safe and effective ECT of cutaneous and subcutaneous tumors [8], [9]. ESOPE proposes the treatment of deep and small tumor diameter (less than 4 mm) with needle electrodes with 4 mm gap between them (Type II electrode). Needle electrodes generally need to be inserted deeper than the deepest part of the tumour [10]. However, the authors simulated without the tissue electroporation model. For small and superficial nodules, the procedure suggests plate electrodes (Type I electrode).

The largest contact between tumor and electrodes provides more homogeneous local electric field distribution and improves the efficiency of electrochemotherapy [11]. The plate electrodes require different setups on equipment dependent on tumor diameters. This setup interferes with the treatment application when the patient has multiple nodular lesions of various sizes. Some works use conductive gel between plate electrodes and tumors to reduce the empty spaces. This methodology provides homogeneous electric field inside the tumors of different sizes [12]–[15]. Ivorra *et al*. [5] demonstrated a good gel conductivity is about 0.5 S/m with plate electrodes, the tumor diameter model was 4 mm. However, the plate electrodes were used for superficial tumors. Clinical trials on patients have revealed that tumors can be less than 3 mm or even greater than 30 mm in size [1], [16], [17].

The electrochemotherapy with plate electrodes needs individual tumor configuration of electrode distance and electric potential. The needle electrodes and electric potential applied are fixed, independent of tumor sizes. The gel eliminates the problem of electrode-tumor contact [5]. Gel and plate electrodes improve the treatment of multiples superficial nodules with different sizes. However, needle electrodes and gel may be providing a better tumor free margin than plate electrode and gel. An investigation about this procedure is necessary.

Squamous Cell Carcinoma (SCC) is a malignant skin tumor common in dogs and cats. The dog cutaneous SCC shows some clinical findings: the mean age affected is about 8 years; sunlight may induced tumors in unpigmented or lightly pigmented skin, such as the abdomen [18]. There are studies of electrochemotherapy treatment of SCC in cats [12], [19]–[21]. However, there are few reports about this same treatment in dogs. The incidence of this tumor is higher in cats, accounting for 15- 49% in cats and 2-15% of all cutaneous tumors in dogs [12].

The purpose of this study was to investigate the electrochemotherapy of small cutaneous tumors (≤4 mm) using needle electrodes and conductive gel. The numerical tumor model was validated with an electrochemotherapy treatment in canine SCC.

Materials and Methods

In vivo Electrochemotherapy

A 9-year-old female stray dog, 19 kg, had an ulcerated formation of SCC. There were numerous verrucous lesions, ranging from 3 mm to 25 mm in diameter, on the abdominal region, extending to the medial face of the right hind limb, and on the region of distal femur. Other clinical signs were not observed. The surgery margin recommended for SCC is 10-30 mm for most lesion [22]. In this patient, the surgery was not recommended because of the tumor extension.

The animal was anesthetized and injected with intravenous bleomycin (15 000 IU/m2) before the application of electric pulses. Electric pulses of 130 kV/m, 8 pulses, 100 μs, and 10 Hz were generated with a BTX ECM 830 (Harvard Apparatus,

Holliston, MA, USA) and were delivered in a single session using needle electrodes. The electrode has two pairs of three parallel-aligned arrays, the distance between electrodes is 5 mm, as shown in Figure 1. The electric fields were defined to plate electrode in ESOPE (7). After the treatment, the patient was clinically monitored during 373 days; there was no cancer recurrence. The treatment was performed in agreement with the recommendations of the animal ethical committee.

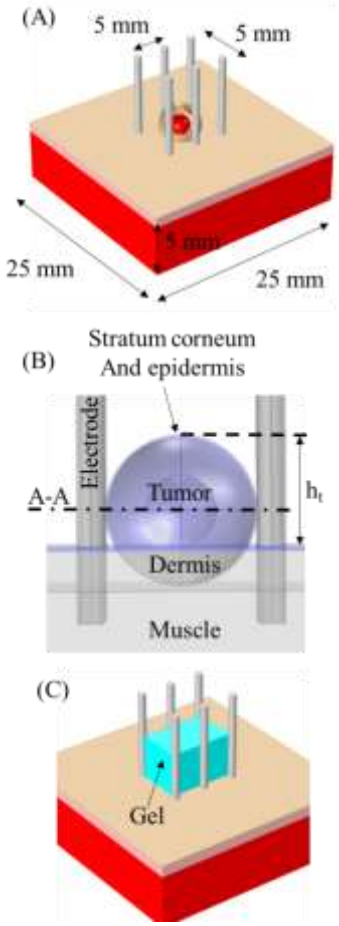

Figure 1. Geometry model of cutaneous tumor. (A) 3D model, needle electrode diameter is 0.7 mm. The cut surface represents epidermis and stratum corneum; the solid sphere is the tumor enveloped by dermis (translucent). (B) Tissue description, dot-trace line is the cutting plane A-A, h_t is tumor height above the skin. (C) The gel model on the simulation.

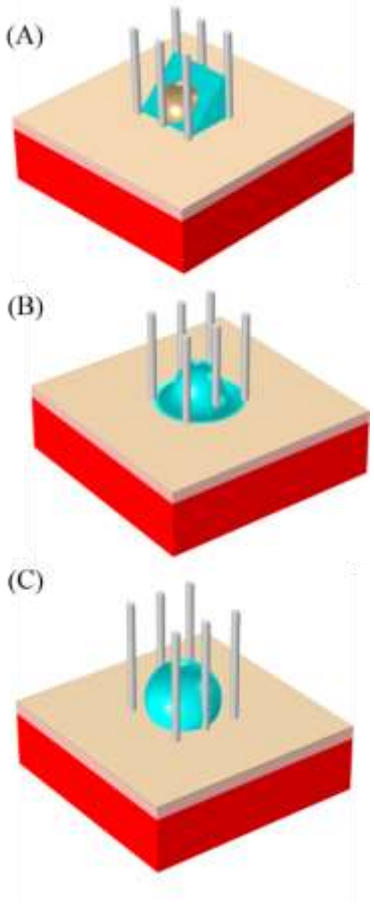

Figure 2. Three cases in which the geometry of the gel is not optimal. (A) The geometry along the axis between electrode lines is not constant and does not cover all tumor; this condition should be avoided. (B) Gel spreads outside the tumor region. (C) Uniform layer on the tumor surface.

Electroporation Model on Tissue Properties

The five simulations describe the electrode inside the tissue of 1 mm thickness, one simulation touches the skin tumour; every simulation shows a tumor diameter smaller than the minimum electrodes distances. The tumor height above the skin are 2 mm and 3 mm. The needles diameter is 0.7 mm. The model of cutaneous tumor

consisted of four tissues: epidermis and stratum corneum (SC), dermis, tumor and muscle. The tissue model was studied by Suzuki *et al*. [4] with experimental analyses of electrochemotherapy and thickness tissues values applied for dogs. Epidermis and SC thickness is 0.06 mm, dermis thickness is 1 mm, muscle thickness is 5 mm, and tumor diameters are 2 mm and 1 mm for Figure 3 (C) simulation. The gel conductivity is 0.5 S/m; this value was proposed with plate electrodes [5]. Model geometries of gel deposition are shown in Figure 2. The electric field applied to all simulations is 130 kV/m.

The microscopic effect of electroporation is the increase of membrane conductivity. The macroscopic effect results on tissue conductivity dependence of electric field. Researches do not take into account this feature [5], [11], but it has significant alteration on electric field distribution on electrochemotherapy planning [4], [5], [3]. The simulations in this work have been performed assuming that electroporation increases the tissue conductivity. The sigmoid function of conductivity dependence with electric field is described by [23]:

$$\sigma(E) = \sigma_0 + \frac{\sigma_{max} - \sigma_0}{1 + D.e^{-\left(\frac{E-A}{B}\right)}} \tag{1}$$

$$A = \frac{E_{irrev} + E_{rev}}{2}$$

$$B = \frac{E_{irrev} - E_{rev}}{C}$$

where σ_{max} is the maximal conductivity of permeabilized tissue, E_{rev} and E_{irrev} are reversible and irreversible threshold of electric field, respectively, and $C = 8$ and $D = 10$ are sigmoid function parameters [23]. The values of σ_0, σ_{max}, E_{irrev} and E_{rev} for each tissue are presented in Table 1.

TABLE 1.
Electric parameters of electric conductivity dependency of tissue [6].

	$\boldsymbol{\sigma_0}$ (**S/m**)	$\boldsymbol{\sigma_{max}}$ (S/m)	$\boldsymbol{E_{rev}}$ (**kV/m**)	$\boldsymbol{E_{irrev}}$ (**kV/m**)
Epidermis and SC	0.008	0.800	40	120
Dermis	0.250	1.000	30	120
Muscle	0.135	0.340	20	80
Tumor	0.300	0.750	40	80

In results, the values of *E* are displayed from E_{rev}=40 kV/m to E_{irrev}=100 kV/m. The black color represents $E < E_{rev}$ (no effect); gray color, $E_{rev} < E < E_{irrev}$, effective electrochemotherapy treatment (if drug quantify is sufficient in the treated tissues [1]); white color, $E > E_{irrev}$, irreversible electroporation.

Numerical Modeling

The tumor dimensions and electric parameters of simulation were obtained from experimental application. The electroporation tissue model was implemented on numerical model. Finite-element method (FEM) divides the geometric model into a finite number of subdomains. In these subdomains, the differential equations related to the phenomena under study are solved. For electric field distribution, the differential equation for steady electric current in each subdomain (volume conductor) is based on

the Laplace equation. The boundary conditions were Neumann's condition on the external surfaces (all insulating). The contact between the electrode and the tissue was modelled as Dirichlet's boundary condition.

FEM simulations using the COMSOL Multiphysics software package (4.4 package update 2, COMSOL, Inc., Burlington, MA, USA) computed the numerical solution of partial differential equations. The mesh of tetrahedral elements was generated by the FEM tool; the result mesh was refined until the difference in numerical solution was less than 0.5%. The mesh of the models varied between 105,173 and 151,124 elements.

The model simulation was run on a personal computer (Intel Core i5-2500, 3.3GHz CPU, 4 GB RAM) with Windows 7 (x64, Microsoft, Inc., Redmond, WA, USA) operating system.

Results

Figure 3 presents dog abdomen with a lot of SCC tumors before (A) and after (B) the electrochemotherapy treatment. The circle indicates the tumor model of 4 mm. The validation compares this tumor treatment result (tumor was eliminated with one session with needle electrodes) and the *in silico* tumor model.

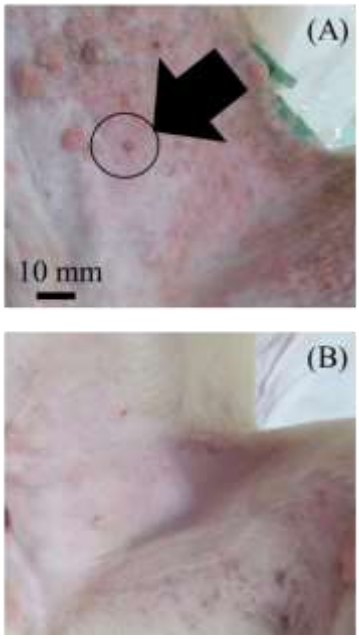

Figure 3. Electrochemotherapy treatment with needle electrode of SCC. The circle presents the tumor diameter of 4 mm modeled in this study. Pulses of 130 kV/m, 8 pulses, 100 µs, and 1 Hz. (A) Before the treatment. (B) 9 months after the treatment, the animal showed complete remission of the disease. The tumor diameter is smaller than the distance between the electrodes.

Figure 4 shows the simulated electric field magnitude distribution for subcutaneous tumor treated with needles. The black color represents $E< E_{rev}$, gray color, $E_{rev}<E<E_{irrev}$, white color, $E> E_{irrev}$. The electrochemotherapy treatment is effective when $E_{rev}<E<E_{irrev}$. Figure 4 (A) and (B) present simulation results with tumor height, h_t, above the skin of 2 mm and 3 mm, respectively. The conductive gel (σ_{gel}=0.5 S/m) is applied on tumor with h_t=3 mm in Figures 4 (C). The tumor in Figure 4 is smaller than the distance between electrodes. The three cases result in non-homogeneous electric field distributions. The tissues with low conductivity (epidermis)

are affected by higher electric fields, the electroporation models (membrane conductivity) is directly proportional to the electric fields. Because of this, the electroporation tends to produce uniformity on tissues conductivity.

The ideal gel deposition covered all tumors with a homogeneous layer. In Figure 4 (C), the gel geometry is ideal, but so far from reality. Figure 5 presents three non-ideal situations with different gel geometries. Figure 5 (A) shows a situation to be avoided, because part of the tumor is not covered by the gel. Figure 5 (B) presents a realistic situation; the gel spreads on the tumor and it is immediate vicinity with different layer thicknesses. In Figure 5 (C), the needle electrodes are not inside the skin and the gel covers just the tumor.

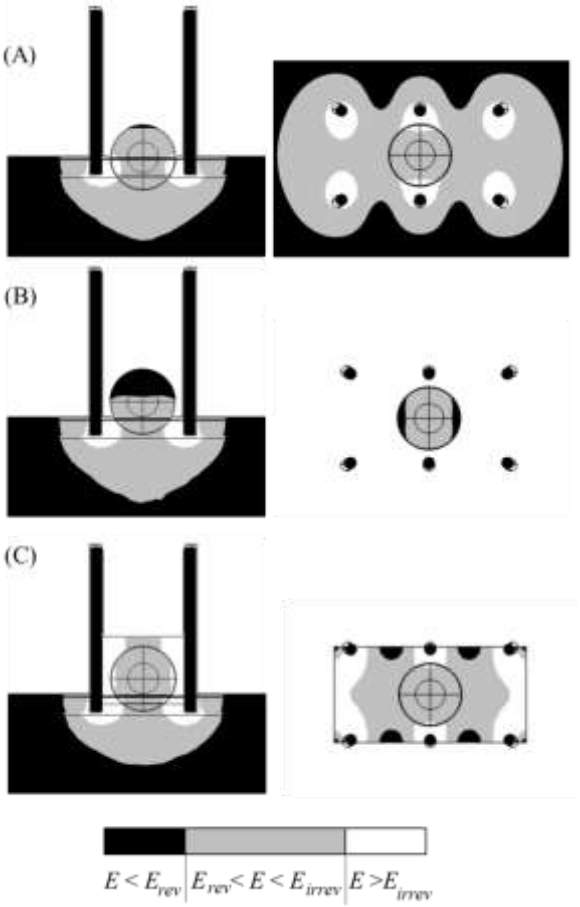

Figure 4. Local electric field distribution. The cross-lines with two spheres represent the dermis and the tumor. Pictures on the left show lateral cross of 3D model and pictures on the right show top cross-sections A-A, Fig. 1 (B), at the dermis+tumour middle. Black color indicates $E<E_{min}$ (40 kV/m), gray color $E_{min}<E<E_{irrev}$ (ideal electric field magnitude for treatment), and white $E>E_{irrev}$ (100 kV/m). Electric field applied is 130 kV/m. (A) Tumor is 4 mm, distances between electrodes are 5 mm, h_t=2 mm. (B) h_t=3 mm, without gel. (C) h_t=3 mm, σ_{gel}=0.5 S/m.

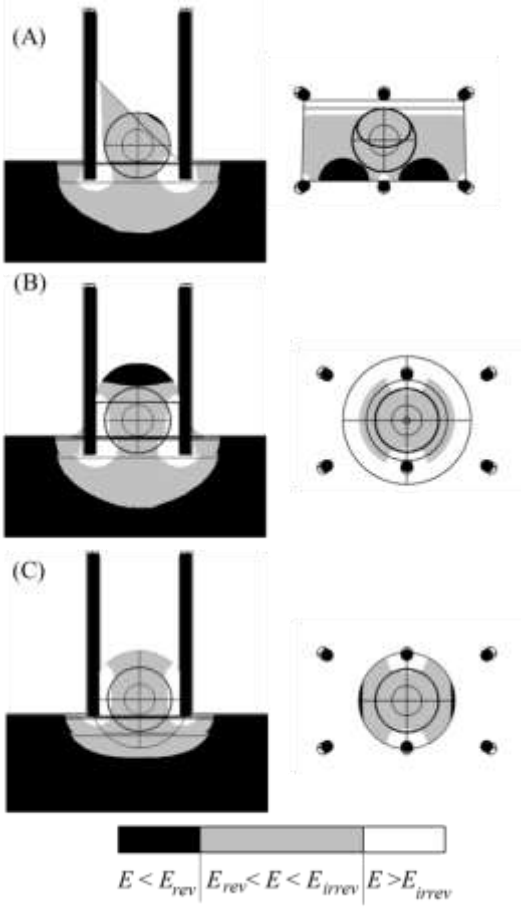

Figure 5. Local electric field distribution with three different non-optimum gel geometries. Black color indicates $E<E_{min}$ (40 kV/m), gray color $E_{min}<E<E_{irrev}$ (ideal electric field magnitude for treatment), and white $E>E_{irrev}$ (100 kV/m). Electric field applied is 130 kV/m. (A) Tumor is not properly covered with gel (σ_{gel} = 0.5 S/m). (B) Gel surface on top of the tumor is slightly convex, irregular and spread over the epidermis. (C) The gel layer covering the whole tumour is thick enough, and the electrodes are not inside the skin.

Discussion

The electrochemotherapy protocol from ESOPE proposed 8 electric fields of 130 kV/m, 100 μs, 5 kHz [8]. The sigmoidal model (Eq. 1) ignores the effects caused by multiple pulses, different frequency and length pulses. However, this study presents a methodology with similar clinical results of electrochemotherapy treatment. Studies have demonstrated that *in silico* methodologies can predict clinical results of electrochemotherapy [4], [5], [24].

We focus on this methodology on the success and safety of electrochemotherapy treatment. The model showed this idea with threshold values (E_{rev}=40 kV/m and E_{irrev}=100 kV/m) used to produce graphic results. Because of this, the irreversible electroporation limit (E_{irrev}) shows excessive tissue damage area larger than the experimental results [4]. E_{irrev} presents variation between, tissues and models, 45 kV/m (muscle) [25] to 173.3 kV/m [5]. This threshold value of E_{irrev} predicts more damage area on the dermis and epidermis than muscle and tumor tissues. E_{rev} is more conservative, all tissues are electroporated to produce gray scale.

The *in vivo* electrochemotherapy treatment was simulated in Figure 4(A). The simulation argues with the clinical result, the small tumor (4 mm) was eliminated even using needle electrodes. Although Figure 4(A) shows tumor elimination, it does not happen when tumor height is 3 mm or higher (Figure 4 (B)).

The ESOPE suggests the utilization of plate electrodes with 4, 6 and 8 mm gap distances to treat small superficial tumour. Nevertheless, this treatment recommends ensuring the largest electrode-tissue contact surface [4], [11]. Superficial tumors less than 4 mm in diameter have small volumes complicating the success of electrochemotherapy. Note that in Figure 4(B), the needle electrode did not eliminate the tumour. If we use plate electrode, with the same distance and applied electric potential, the tumor can be eliminated [4]. The use of plate electrode for superficial tumour instead of needle electrodes occurs due to the best contact between plate electrodes. However, the conductive gel produces the same effect of this best contact even when using the needle electrodes. Figure 4(C) shows this effect.

The clinical use of electrochemotherapy on multiple tumors shows the worst outcomes in terms of the amount of time spent. There are tumors of different sizes; it is difficult in veterinary to measure the distance between plate electrodes to apply the adequate electric field. In this case, the needle electrodes are more practical and easier. The electric field is fixed and the tumors of different sizes are eliminated with the same electrodes and gel.

Figure 4 (C) shows the advantage of correct gel utilization. The electric field distributions are uniform and enough for tumor elimination with electrochemotherapy. Moreover, tumor diameters less than 4 mm (tumor volume less than 0.03 cm^3) can be eliminated by gel and needle electrode. Even the distances between needles are larger than the tumor diameter.

Some authors use the gel to avoid empty spaces between plate electrodes and tissues [12], [13]. These authors used commercial gels (gels for electrocardiogram or ultrasonography) [14], [15], [26]. Ivorra *et al*. [5] show that 0.5 S/m is the ideal electric conductivity of the gel. The commercial gels do not present electric conductivity and sterility adequate to electrochemotherapy. This gel can be made with agar and sodium chloride [7]. In this study, even the gel with 0.5 S/m with needle electrodes was adequate.

The ideal gel deposition is homogeneous, thick enough to cover the entire tumor, Figure 4(C). However, this case is not realistic; the clinical use of gel may result in practical mistakes. The simulations in Figure 4 demonstrated that electrochemotherapy is more robust with the gel. Even with the inappropriate gel deposition, the result is an efficient treatment. The gel does not cover part of the tumor in Figure 5 (A). Therefore, the local electric field is enough to produce an efficient treatment. This situation must be avoided. Figure 5 (B) presents non-homogenous gel layer on the tumor. The thin gel layers near the electrode reduce the current passage to superior areas of tumor; consequently, there is a small local electric field in the tumor top. It is interesting to note that the gel spreading on tumor base does not increase the depth effect of the local electric field. The effective treatment is limited inside the needle electrodes. Despite a layer of gel around the tumor and the needle electrode being not inside the skin as shown in Figure 5 (C), the tumor is eliminated.

The mistakes on gel deposition do not invalidate the technique. Moreover, this study demonstrated that even with fail deposition, the tumor was eliminated. On the other hand, we recommend that the needle electrodes must be inserted on skin to eliminate (possible) infiltrating tumor cells on muscles. The spreading gel does not increase the distribution of electric field on deeper tissues (muscle).

Ivorra *et al.* [5] observed that the average current for conductive gel group with plate electrode tripled the average current for without gel group. This effect was observed in our simulations (data not shown). On the other hand, it is interesting to note that the current drawn from the generator using gel with needle electrodes increase by less than 10%.

In this work, the electrochemotherapy with needle electrodes of small superficial tumours was studied to improve the treatment. The conductive gel with needle electrodes was indispensable for homogeneous electric field distribution into tumour and, consequently, effective tumour elimination. We recommend that needle electrodes may be under the skin to provide a tumour free margin under the superficial tumour (Figures 5 (B) and (C)).

Conclusions

The conductive gel is essential for successful treatment of superficial tumour with needle electrodes. The needle electrodes provide a free margin under the tumour. The advantages of using gel and needle electrodes are more clinical practical and decrease of medium current in comparison with plate electrode.

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