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Robustness of Treatment Planning for Electrochemotherapy of Deep-Seated Tumors

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Abstract Treatment of cutaneous and subcutaneous tumors with electrochemotherapy has become a regular clinical method, while treatment of deep-seated tumors is still at an early stage of development. We present a method for preparing a dedicated patient-specific, computer-optimized treatment plan for electrochemotherapy of deep-seated tumors based on medical images. The treatment plan takes into account the patient's anatomy, tissue conductivity changes during electroporation and the constraints of the pulse generator. Analysis of the robustness of a treatment plan made with this method shows that the effectiveness of the treatment is not affected significantly by small single errors in electrode positioning. However, when many errors occur simultaneously, the resulting drop in effectiveness is larger, which means that it is necessary to be as accurate as possible in electrode positioning. The largest effect on treatment effectiveness stems from uncertainties in dielectric properties and electroporation thresholds of treated tumors and surrounding tissues, which emphasizes the need for more accurate measurements and more research. The presented methods for treatment planning and robustness analysis allow quantification of the treatment reproducibility and enable the setting of suitable safety margins to improve the likelihood of successful treatment of deep-seated tumors by electrochemotherapy.

Keywords Electrochemotherapy · Electroporation · Treatment planning · Deep-seated tumor

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Introduction

Electrochemotherapy (ECT) is a treatment in which a specific chemotherapeutic drug having an intracellular target is combined with a strong pulsed electric field that increases cell membrane permeability—electroporation (Orlowski et al. 1988; Sersa et al. 1995). This increases the amount of molecules that enter cancer cells and have a cytotoxic effect. To achieve a complete response of the treated tumors, the electric field used for electroporation has to exceed a threshold value in the entire tumor volume (Miklavcic et al. 2006a; Sersa et al. 2008). In the last decade ECT has been successfully used for treatment of cutaneous and subcutaneous tumors, mainly melanoma (Campana et al. 2009; Marty et al. 2006). The success of ECT, its clinical applicability and recent development of more powerful electric pulse generators and new electrodes have resulted in the first clinical uses of ECT for treatment of deep-seated tumors (Miklavcic et al. 2010).

In the European Standard Operating Procedures of Electrochemotherapy (ESOPE) study a standard operating protocol was developed for ECT of cutaneous and subcutaneous tumors that provides physicians with a set of appropriate electrodes and electric pulse parameters depending on tumor size and location (Mir et al. 2006). This protocol, however, cannot be used for ECT of deep-seated tumors because of increased treatment complexity. Deep-seated tumors can be much bigger than cutaneous or subcutaneous

ones; their shape can be irregular; they can be in the vicinity of vital organs, damage to which has to be avoided; and the electric properties of the surrounding tissue as well as of the tumor can vary significantly. Since it is necessary to cover the entire volume of the tumor with electric field above the threshold to achieve a desirable effect, the choice of electrode position and voltages applied between the electrodes varies from case to case, which is why an individualized treatment plan, similar to radiotherapy treatment plans, is necessary (Bortfeld 1999). As the electric field distribution inside the target tissues is one of the most important predictors of electroporation (Miklavcic et al. 1998), the use of numerical models of electroporation has been proposed, in combination with optimization algorithms, as a means of ECT treatment planning (Corovic et al. 2008; Sel et al. 2007).

The treatment plan should be robust enough to prevent uncertainties both in the treatment planning stage and in the treatment itself from influencing the treatment outcome. The uncertainties include (1) all the input parameters for the numerical model (conductivity values for each tissue, electroporation thresholds and precision of the anatomical model) and (2) the difficulty in precisely positioning the electrodes (relative to each other and relative to the tumor). These uncertainties have to be carefully analyzed, and their significance for the success of the ECT treatment has to be evaluated.

Here, we present a method for creating a dedicated patient-specific treatment plan for deep-seated tumor ECT, its application on a case of melanoma metastasis in the thigh and a qualitative assessment of the treatment plan robustness.

Methods

Assembling a Patient-Specific Numerical Model

The first step in ECT treatment planning is the construction of a sufficiently detailed patient-specific model of the anatomy. Medical images (CT) of the region of interest were first segmented and then used to build a 3D geometry by approximating the segmented tissue with a closed spline curve and connecting the curves in the third dimension using Matlab (Mathworks, Natick, MA) as described previously (Sel et al. 2007). This geometry was then imported into COMSOL Multiphysics (COMSOL, Stockholm, Sweden), where finite-element analysis was performed.

In the presented case (Miklavcic et al. 2010) the geometry consisted of a melanoma metastasis in the right thigh, two nearby muscles (sartorius and gracilis) and surrounding adipose tissue. After the tissue geometry was built, the electrodes chosen for the treatment were added (in the presented case, four stainless-steel needle electrodes with a 30-mm exposed tip and the rest of the length insulated were used) (Fig. 1). As the skin is penetrated by the needle electrodes, its high impedance does not affect the calculations; therefore, it was not considered in the model along with other tissues located away from the tumor (e.g., femur and other thigh muscles). At the location of these tissues, the electric field strength is low and not significant for the treatment.

In the presented model, tumor, muscle and adipose tissue were modeled; their bulk conductivities were taken from the literature (0.135, 0.75, 0.2 and 0.02 S/m, for muscle in the direction perpendicular to muscle fibers, muscle in the direction parallel to muscle fibers, tumor and adipose tissue, respectively) (Gabriel et al. 1996; Haemmerich et al. 2009). These values present conductivities at a frequency of 10 or 50 Hz and have been previously used to accurately predict tissue electroporation and the total current delivered during electric pulses (Pavselj et al. 2005).

The mathematical model of electroporation used in the study is described in more detail by Sel et al. (2005). Briefly, the Laplace equation for static electric currents was used to calculate the electric field distribution in the model. A sequential model of electroporation was used, which takes into account the

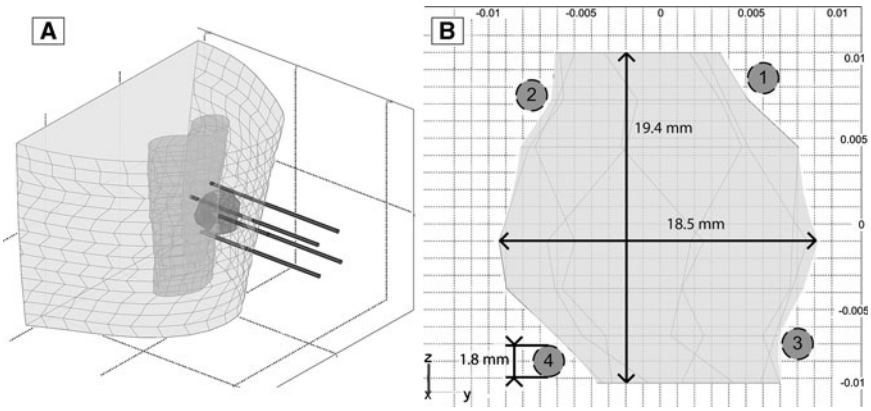

Fig. 1 **a** Model geometry. Four electrodes are inserted into the thigh (*light gray*) around the tumor (*dark gray*) according to the treatment plan. Also shown are two adjoining muscles that the electrodes penetrate by a few millimeters. **b** Electrode positions in the cross section perpendicular to the electrodes' axis, through the center of mass of the tumor

conductivity changes during electric pulse delivery due to electroporation (Cukjati et al. 2007; Ivorra et al. 2009). In the model, electroporation-increased tissue conductivities were increased by a factor of 3.5 (Cukjati et al. 2007) and the reversible thresholds for electroporation were considered to be 400 V/cm for tumor tissue, 100 V/cm for adipose tissue and 200 and 80 V/cm for muscle tissue in the direction perpendicular to muscle fibers and in parallel direction, respectively (Miklavcic et al. 2000; Corovic et al. 2010). The model assumes that the conductivities return to their initial values before the next pulse is delivered and does not differentiate between reversible and irreversible electroporation. Furthermore, the model considers only one pulse between each electrode pair because in order to model conductivity changes between each successive pulse in the regular eight-pulse train, the time course of conductivity after a pulse would have to be known.

Treatment Planning

The assembled numerical model was used together with a genetic algorithm, as previously described, to provide an optimal treatment plan (Corovic et al. 2008; Zupanic et al. 2008). When setting up the optimization, constraints had to be taken into account, e.g., feasible positions of the electrodes and specifications of the pulse generator (Cliniporator Vitae^{TM}; IGEA, Carpi, Italy). Two treatment plans were generated: In the first, the position constraints were that the electrodes should be outside the tumor but $<$1 cm away, while in the second, there was an additional fifth electrode inserted in the center of the tumor. A lower number of electrodes did not yield a successful solution, and more were considered too difficult to position correctly. Due to problems with insertion of the central electrode during the actual treatment, the four-electrode plan was finally adopted (Miklavcic et al. 2010). The pulse generator constraints were the maximum available voltage (3,000 V) and current (50 A). In addition, only two electrodes at a time can have a set potential during electric pulse delivery. In the presented case the algorithm searched for the optimal positions of each of the four electrodes and the optimal voltage between each pair of electrodes. The optimization algorithm was set to maximize the volume of the tumor covered with electric fields over the reversible electroporation threshold and reduce the volume of nearby healthy tissue covered with fields over the irreversible electroporation threshold (Davalos et al. 2005; Rubinsky et al. 2007). Since the tumor coverage is essential for successful treatment, it was given 10 times more weight than damage to healthy tissue. The final number of parameters optimized for the treatment was 15: depth of insertion of all electrodes, y and z positions of each electrode and voltage between each pair of electrodes.

Robustness Analysis

To assess the robustness of the presented treatment plan, we used the same numerical model as in the treatment planning and calculated the volume of tumor covered with an electric field over the reversible electroporation threshold, while varying a single model parameter at a time. In this parameterization study the parameters analyzed were the model inputs that were taken from the literature, such as electrical conductivity values and reversible electroporation thresholds for each tissue as well as the treatment planning parameters acquired from the optimization (electrode positions and voltages).

Every parameter was varied in five steps from the optimal position, and the percentage of tumor volume coverage was determined each time. Electrode positions perpendicular to the axis of insertion were varied in 0.5-mm steps away from the tumor in two perpendicular directions (y, z), depth of electrode insertion was varied in 1-mm steps in both directions (deeper and shallower penetration than optimal), voltages were varied in steps of 100 V below the optimal values, electrical conductivities were varied in steps of 10% of the values used in the model in both directions (higher and lower values than those used in the model) and electroporation thresholds were varied in steps of 50 V/cm above the values used in the model. All parameters, their baseline values and ranges are summarized in Table 1. The chosen parameters represent the uncertainties in the treatment procedure (positions) and tissue parameters (tissue conductivities and electroporation thresholds), while voltage can vary by up to 3% over the entire output voltage range (i.e., up to 90 V at the maximum 3 kV output). An additional motivation for investigating reductions in voltage was to verify the appropriateness of the optimization step and to determine if any electrode pair plays a critical role in ensuring total tumor coverage. Furthermore, it could be necessary to reduce the voltage in the clinical setting if the pulse generator is unable to provide the required current output due to technical limitations. This approach highlights the most critical parameters for the success of ECT and can also serve in determining the safety factors needed for treatment and predicting the ECT outcome.

Results and Discussion

We applied a genetic algorithm to optimize the positioning of four electrodes outside the tumor and necessary minimum

Table 1 Summary of varied parameters, their respective baseline values and the ranges in which they were varied in the analysis

Parameter	Range	Baseline value
Depth of all electrodes	−5 to +5 mm	Treatment plan (Fig. 1)
Single electrode position	0–2.5 mm away from tumor	Treatment plan (Fig. 1)
Voltage between each electrode pair	0 to −500 V	Treatment plan (Fig. 1)
Tumor electroporation threshold	400–650 V/cm	400 V/cm
Fat conductivity	0.01–0.03 S/m	0.02 S/m
Tumor conductivity	0.1–0.3 S/m	0.2 S/m
Muscle conductivity x, y direction	0.075–0.203 S/m	0.135 S/m
Muscle conductivity z direction	0.375–1.125 S/m	0.75 S/m

Total number of parameters varied: 19

voltage to apply between each electrode pair. Current drawn from all electrode pairs was calculated, and we established that it was below the maximum 50-A current limit of the Cliniporator Vitae electroporation pulse generator. The optimized treatment plan was successful in covering the whole tumor volume with an electric field of no less than 400 V/cm, the estimated tumor electroporation threshold value; the details of the treatment plan are presented in Figs. 1 and 2 and Table 2. Figure 2 shows tumor coverage after each pulse application; it can be seen that the whole tumor is covered after the application of pulses to all six electrode pairs. It would be possible to cover the whole tumor with just two electrodes; however, the required voltage would be much higher, and the amount of healthy tissue damage would increase significantly. An additional benefit of using multiple electrodes is that many parts of the tumor are covered more than once and with different directions of the electric field, which has been shown to increase electroporation efficiency and molecular uptake (Rebersek et al. 2007).

According to our criteria, a solution is maximally fit when complete tumor coverage has been achieved and the volume of surrounding tissue above the irreversible electroporation threshold has been reduced to a minimum. In the case of using just two electrodes, a maximally fit solution would be only marginally robust. Although robustness (of tumor coverage) could be increased, by, e.g., increasing the applied voltage, such an increase would be at the expense of fitness. In the case where there are more electrodes used, even a maximally fit solution (therefore the most sensitive to errors) is inherently more robust than using only two electrodes because some parts of the tumor are permeabilized more than once (Sersa et al. 1995).

Table 2 Voltage between each electrode pair according to the optimized treatment plan

Electrode pair	Voltage (V)
1–2	1,100
1–3	1,600
2–4	1,600
3–4	1,100
1–4	1,800
2–3	1,900

In Figs. 3 and 4 we show the results of the robustness analysis. Lowering the voltage on one electrode pair by 300 V does not affect the tumor coverage at all (Fig. 3), while decreasing the voltage by 500 V causes a small volume (ranging 0.05–0.2%) to be below the threshold. This can be explained by the fact that most of the tumor

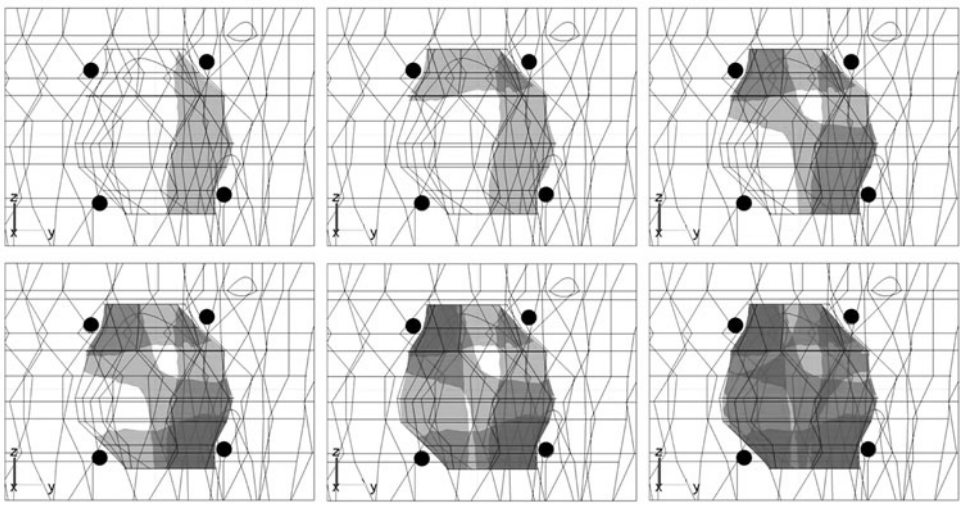

Fig. 2 Tumor electroporation after application of each pulse in the sequence. *Gray areas* show where the electric field exceeded the threshold value

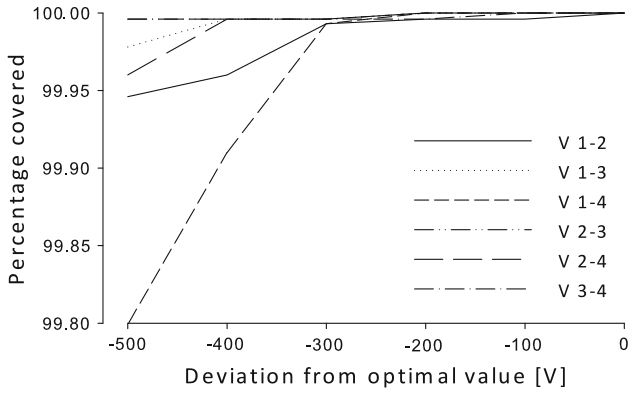

Fig. 3 Robustness analysis: dependence of tumor coverage with an electric field over the electroporation threshold on different parameters. Effect of reducing voltage on a single electrode pair in steps of 100 V

volume is covered by more than a single pair of electrodes (Fig. 2). Therefore, a drop in effectiveness of one electrode pair does not affect the end result as dramatically as might be expected. The results also suggest that 100 V, which is also the smallest adjustment step in the Cliniporator Vitae clinical pulse generator, for which this treatment plan was developed, is an appropriate step in voltage optimization of ECT.

The highest drop in tumor coverage was observed when increasing the tumor electroporation threshold (Fig. 4a); increasing the threshold to 650 V/cm reduces tumor coverage to just above 45%. The electroporation threhsold is a critical parameter in many aspects. First, the thresholds are different for different tissues, and this fact is mostly attributed to differences in cell shapes and sizes between the tissues (Cemazar et al. 1998). Threshold measurements have so far been scarce (Miklavcic et al. 2000) and are further complicated by the fact that many tissues, among them larger tumors, are inherently heterogeneous and therefore probably exhibit a range of thresholds instead of just one. Furthermore, it is hard to cover a large volume of tissue with very high electric fields as the voltages and currents required would be higher than available from commercial pulse generators. All this calls for more research into the tissue electroporation thresholds and development of statistical

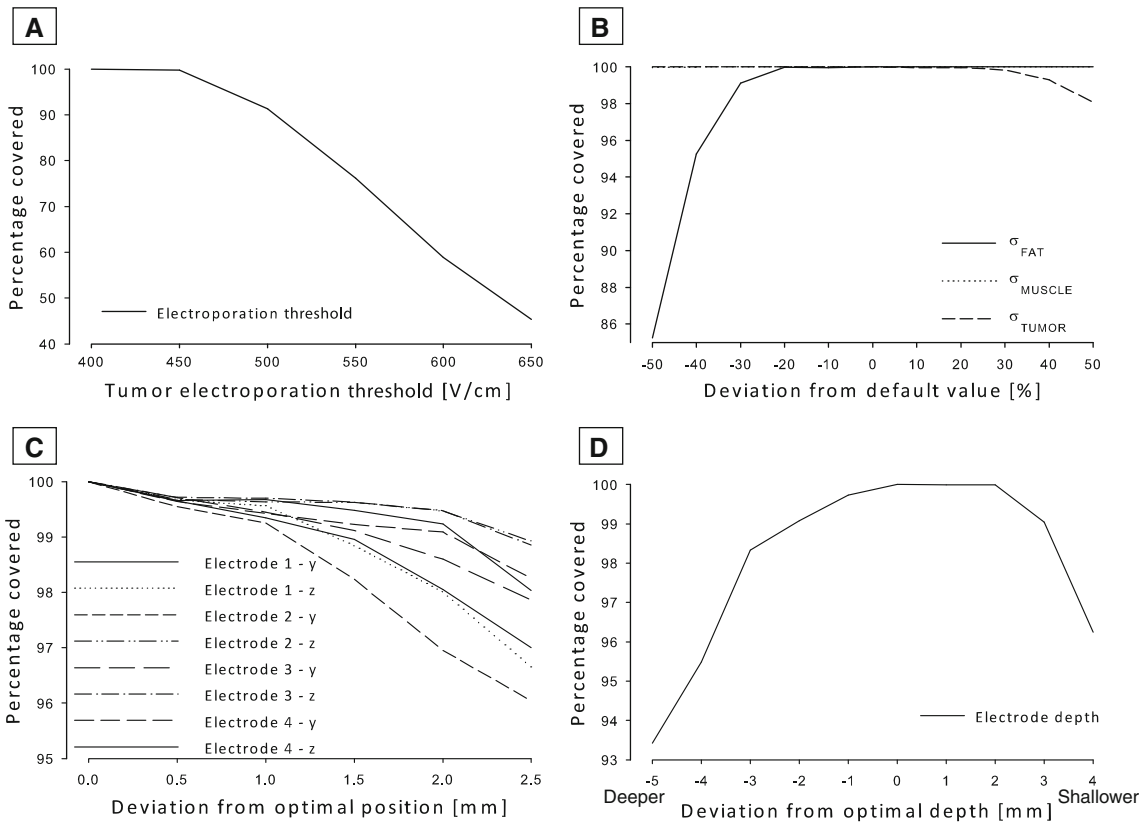

Fig. 4 Robustness analysis: dependence of tumor coverage with an electric field over the electroporation threshold on different parameters. **a** Effect of deviations of tumor electroporation threshold in steps of 50 V/cm. **b** Effect of deviations of tissue conductivities from values used in treatment planning in steps of 10% of the values used. **c** Effect of errors in electrode positions along a single axis in steps of 0.5 mm away from tumor surface. **d** Dependence on depth of insertion of all electrodes. Note that all plots do not share the same vertical scale

models of tissue electroporation (Goldberg and Rubinsky 2010) to improve the efficiency of ECT.

Deviations in tissue conductivity also affected the tumor coverage (Fig. 4b). More precisely, it is the ratio of conductivities between the tumor and surrounding (in this case adipose) tissue which is the (most) critical factor. When the conductivity of adipose tissue is lowered or, alternatively, the conductivity of the tumor is increased, the ratio of $\sigma_{TUMOR}/\sigma_{FAT}$ is increased and the treatment effectiveness is reduced significantly. If this ratio is changed from 10 as in the original treatment plan to 20, tumor coverage is reduced to 85%, while if the ratio is reduced, the coverage is not affected but the robustness is increased. This stems from the fact that the lower conductivity of the surrounding tissue acts as an insulator, and it takes a larger part of the voltage between the electrodes according to the voltage divider principle (Pavselj and Miklavcic, 2009), which results in a decreased electric field and less successful electroporation. While tissue conductivities are not parameters that can be controlled by the optimization or the performing physician, it is necessary to note that data regarding low-frequency conductivities of human healthy and cancerous tissues are scarce, are difficult to measure and have values published by different authors/studies that vary significantly (Gabriel et al. 2009; Miklavcic et al. 2006b). It has been reported previously (Neal and Davalos 2009; Daniels and Rubinsky 2009) that inserting electrodes within the target region can greatly improve the electric field within the target region, while at the same time reducing the effect of the unknown conductivity ratio between target and surrounding tissue. When permitted by the nature of the treated tumor and the surrounding tissue, such a practice is a good way of eliminating some of the uncertainties present in this kind of ECT planning.

Electrode positions are also a critical parameter (Fig. 4c, d) since electrode insertion is the part of the procedure that is most prone to errors. Mispositioning a single electrode by 2 mm can already reduce tumor coverage from 100% to <97%. However, if all electrodes miss their target, the results are much more severely affected. When all electrodes are moved away from the tumor in a diagonal direction by 0.7 or 1.4 mm (effectively increasing the distance between the electrodes and the distance between the electrodes and the tumor), the tumor coverage decreases to 87 or 66%, respectively. The depth of insertion is also important, although we assumed at this point that all electrodes were placed at the same depth. Since the needle electrodes used in this case have a 3-cm noninsulated tip (comparable in size to the treated tumor) that delivers the pulses and the rest of the electrode length is insulated, inserting them either too deep or too shallow can cause significant reduction in tumor coverage, e.g., 6% when electrodes are inserted 5 mm too deep (Fig. 4d). These results seem to be in contradiction with previous research (Corovic et al. 2008) that suggested that deeper insertion can be considered safe. However, in the previous calculations of Corovic et al. the tumors' physical size was small in relation to the length of the noninsulated (active) parts of the electrodes, whereas the size of the tumor in the current study was comparable to the noninsulated parts of the electrodes, which increases the possibility of missing the target. Our results suggest that it is necessary to be as accurate as reasonably possible in the operating theater, including use of medical imaging (e.g., ultrasound) for guidance in positioning electrodes. It would be possible to use longer electrodes (with longer active parts) to reduce this sensitivity; however, that would result in a significantly increased current, which the pulse generator might not be able to deliver.

A certain number of errors are likely to be made during the treatment, due to reasons mentioned previously. To ensure that the ECT outcome is not affected by these errors, a suitable safety margin should be employed during the treatment planning stage. The robustness analysis suggests that setting conservative values for dielectric properties (higher $\sigma_{TUMOR}/\sigma_{FAT}$) and higher electroporation thresholds can increase the robustness of the treatment, but care must be taken to avoid excessive electric fields that would cause extensive healthy tissue damage.

Our results show that the presented method of treatment planning for deep-seated tumors by ECT is capable of producing efficient and robust treatment plans in the clinical setting. The robustness analysis indicates that further work is necessary to determine tissue electroporation thresholds and conductivity values, as well as enable accurate electrode positioning during ECT since these two parameters affect the treatment outcome to the highest degree. The presented work sets the ground for numerical treatment planning–based ECT of deep-seated solid tumors, quantifying its reproducibility and enabling the setting of suitable safety margins to improve the likelihood of successful treatment.

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