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Electrode commutation sequence for honeycomb arrangement of electrodes in electrochemotherapy and corresponding electric field distribution

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ABSTRACT

Electrochemotherapy is a treatment based on combination of chemotherapeutic drug and electroporation. It is used in clinics for treatment of solid tumours. For electrochemotherapy of larger tumours multiple needle electrodes were already suggested. We developed and tested electrode commutation circuit, which controls up to 19 electrodes independently. Each electrode can be in one of three possible states: on positive or negative potential or in the state of high impedance. In addition, we tested a pulse sequence using seven electrodes for which we also calculated electric field distribution in tumour tissue by means of finite-elements method. Electrochemotherapy, performed by multiple needle electrodes and tested pulse sequence on large subcutaneous murine tumour model resulted in tumour growth delay and 57% complete responses, thus demonstrating that the tested electrode commutation sequence is efficient.

1. Introduction

Electroporation is a phenomenon that occurs in cell membranes when cells are exposed to sufficiently high electric field [1–4]. Molecules, such as some drugs or nucleic acids, which otherwise are unable to cross cell membrane may then enter cells. This phenomenon is used in combination with some chemotherapeutic drugs e.g. bleomycin and cisplatin for tumour treatment, which is known as electrochemotherapy [5–7]. It is used in clinics for transfer of chemotherapeutic drugs in tumour cells by means of short high voltage electric pulses applied to the tumour [8,9].

Generators of electrical pulses for electroporation are named electroporators. For small tumours electric pulses are delivered to the tissue usually via two metal (plate) electrodes [10,11]. Electrochemotherapy of small tumours is already well investigated and good results are obtained with a single pair of electrodes. To achieve good electrochemotherapy the entire volume of the tumour needs to be effectively permeabilized [12]. On larger tumours pair of electrodes should be repositioned or higher voltage should be used [13]. However, repositioning of electrodes is not practical since positions and amplitudes should be pre-calculated to achieve effective permeabilization over the whole tumour. Moreover, electroporators certified for clinical use do not generate electric pulses with amplitudes over a few kV [14]. Therefore, multiple needle electrodes were suggested for effective tissue permeabilization in electrochemotherapy, i.e. to cover the whole tumour with sufficiently high electric field. Such electrodes allow for treatment of larger tumours and at the same time using lower pulse voltages [15,16].

Electronic circuits which commutate electric pulses between the electrodes are named electrode commutation circuits. Honeycomb and square arrangements of electrodes were mainly suggested proposed as multiple needle arrangements, which theoretically enable the use of infinite arrays of needle electrodes with finite electrode commutation circuit [17]. Therefore the design of electrode commutation circuit does not depend on the number of electrodes but only on the maximum voltage applied and current to be delivered through the electrodes.

The aim of our study was to develop electrode commutation circuit and test its efficiency *in vivo* by performing electrochemotherapy with multiple needle electrodes on larger tumours, which with a single pair of electrodes cannot be achieved. For this we developed an electrode commutation circuit, which commutates the usual electroporation single output signal from an electroporator to multiple electrodes. We used seven-needle electrodes, for which we suggested and tested an effective electrode commutation sequence for tissue electroporation. We also calculated the corresponding electric field distribution in tumour tissue by means of finite elements method (FEM) in 3D model taking into account the increase of tissue conductivity due to electroporation in order to demonstrate also theoretically that the entire tumour volume is exposed to sufficiently high electric field leading to tissue permeabilization and efficient electrochemotherapy.

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2. Materials and methods

2.1. Electroporator (EP-GMS 7.1) with embedded electrode commutation circuit

Both electroporator (EP-GMS 7.1) and embedded electrode commutation circuit for multiple electrodes were developed at the University of Ljubljana, Faculty of Electrical Engineering. The EP-GMS 7.1 electroporator was already used in previously reported studies [18–20]. The main advantage of this electroporator is the ability to automatically commutate electrical pulses between electrodes with embedded electrode commutation circuit.

The user defines electrical parameters of applied electric pulse through the interface of the electroporator (EP-GMS 7.1) on a personal computer (PC). These parameters are then transferred to the executive part of the electroporator. After this transfer the electroporator is ready to generate defined electric pulses in defined sequence.

Electroporator (EP-GMS 7.1) generates square electric pulses from 80 to 530 V, duration from 10 to 1000 μs, repetition frequency from 0.1 to 5000 Hz and from 1 to 32 pulses. Particularity of this electroporator is an embedded electrode commutation circuit which consists of controlling part and executive part (Fig. 1).

The commutation control (Fig. 1) is compatible with external bus interface of microprocessor MCF5204 (FreeScale, USA) or with any other similar 16-bit bus interfaces. Computer board based on microprocessor MCF5204 is a part of EP-GMS 7.1 and it can be controlled by personal computer (PC) via serial port (RS-232). The commutation control works like parallel input–output unit (PIO), which can control up to 38 relays in the executive part of the electrode commutation circuit which corresponds to 19 electrodes. The commutation control registers the time of last command and thus estimates the position of relays. This is necessary due to relatively long switching time of relays. Commutation control functions are designed on Field Programmable Gate Array (FPGA, XCS30-VQ100, Xilinx, USA).

Each executive module (Fig. 1) consists of fourteen relays TRK1703 (Iskra, Slovenia), which with their positions define states of seven electrodes. The first half of relays defines the polarity of electrodes, while the second part defines the impedance state of the electrodes. Each of the electrodes can thus be in one of three possible states: positive, negative or in high impedance. Positive state means that electrode is connected to positive potential of electroporator, negative state means that electrode is connected to negative potential of electroporator and high impedance state means that electrode is disconnected from electroporator. This concept and design of commutating the electroporation signal allows no possibility to short-circuit electroporator.

Maximal voltage of electroporation signal which can be connected to the executive module is 1 kV. Maximal continuous current is 3 A per executive module and 2 A per electrode. Maximal pulse current of maximum duration 10 ms and more than nine times longer pause is 30 A per executive module and 10 A per electrode. Electric (galvanic) separation between the commutation control and electroporation signal is more than 4 kV.

Because of the presence of high voltage and high electric current in the executive module the distance between the contacts and the weight of the anchor in relays is very large. Therefore the switching time of such a relay is relatively long. With proper selection of relays and especially with adequate driving circuit we achieved switching time of 6 ms (Fig. 2). The relay is driven by high voltage Darlington transistors and paralleled by a zener diode. This allowed for optimization of the relay turning off time. The fastest would be without the diode but sooner or later this would result in the transistors break down. Due to ageing and variations in elements, the time reserved for switching was extended to 12 ms. Embedded electrode commutation circuit can therefore automatically commutate electrical pulses between electrodes with frequencies up to 83 Hz.

2.2. Electric field in tumour tissue during the electroporation process

A three-dimensional finite-elements model of tumour tissue (cylinder; diameter: 15 mm, length: 6 mm; Fig. 3) with inserted seven-needle electrodes (honeycomb arrangement; diameter of needles: 0.5 mm, distance between two neighbouring needles: 5.5 mm) was built according to specifications of *in vivo* electrochemotherapy experiment using software package EMAS (ANSOFT Corporation, USA). Applied voltage (±265 V) was modelled as Dirichlet's boundary condition on the surface which presents the cross-section of electrode and

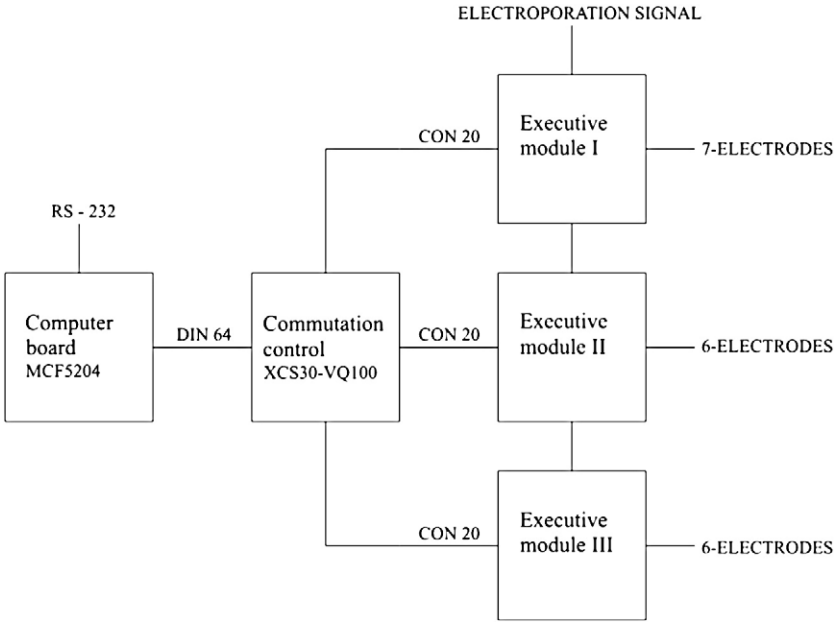

Fig. 1. Block scheme of electrode commutation circuit, which commutates electroporation single output signal from an electroporator to up to 19 independent electrodes. Electrode commutation circuit consists of controlling part (Commutation control XCS30-VQ100) and executive part (three Executive modules) and it can be controlled by personal computer (PC) over serial port (RS-232) on computer board MCF5204.

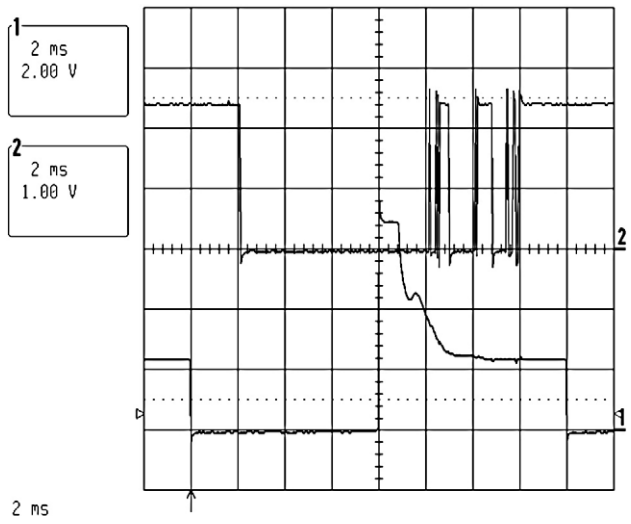

Fig. 2. Switching time of relay Iskra TRK1703. Signal 1 represents negative driving voltage for relay and signal 2 represents the commutation of relay. Signal 2 shows us that coil releases or pulls the anchor in 2 ms and that the anchor then bounces on the contact for 4 ms. Therefore the whole switching time is about 6 ms.

tumour tissue. Dirichlet's boundary condition was also set on the surface of disconnected (high impedance) electrodes to satisfy the conditions, that electrodes are a lot more conductive then tumour tissue. Electropotential of disconnected electrodes was defined as zero, because our model is symmetrical and disconnected electrodes were always in the middle between the connected electrodes. Tumour tissue was mathematically separated from surrounding area by Neuman's boundary condition:

$$J_N = 0, \tag{1}$$

where J_N is the normal electric current density [A/m^2]. The distribution of the electric field intensity in tumour tissue for given electrode geometry was calculated numerically by means of finite-elements method [21]. Tumour tissue was modelled as a quasi-stationary passive and isotropic volume conductor in the quasi-stationary electric current field. A condition in such structure is described by Laplace's equation:

$$\Delta\phi = 0, \tag{2}$$

where ϕ is the electric field potential [V].

Due to a functional dependency of tumour tissue conductivity on electric field intensity, a sequence analysis application for modelling of electrical properties changes during electroporation process was used. In each static model of the sequence analysis tissue conductivity was determined based on electric field distribution in previous model of the sequence analysis:

$$\sigma(k) = f(E(k-1)), \tag{3}$$

where σ is the tissue conductivity [S/m], E is the electric field intensity [V/m], k is the sequential number of static model in the sequence analysis and f functional dependency of tumour tissue conductivity on electric field intensity, which was obtained from previously reported studies [22,23].

In *in vivo* electrochemotherapy experiment we used an electrode commutation sequence as presented on Fig. 4. In the commutation sequence used first (Fig. 4b) all outer electrodes were activated and neighbouring needles were of the opposite polarity (±265 V), while the middle electrode was in high impedance state (0 V in the finite-elements model). After the commutation the second part (Fig. 4c) was delivered in which all outer electrodes were positive (+265 V) and the inner electrode was negative (−265 V).

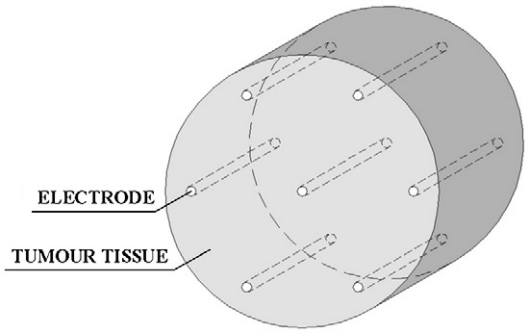

Fig. 3. A geometry of 3D-model of multiple needle electrodes inserted into the tumour tissue. Tumour tissue is in the shape of a cylinder with a diameter of 15 mm and length of 6 mm. Inserted seven-needle electrodes are in honeycomb arrangement with a diameter of 0.5 mm and the distance between two neighbouring needles is 5.5 mm.

The course of electrical conductivity changes inside the model of the tumour tissue due to electroporation is presented in Fig. 5b, with corresponding distribution of electric field intensity presented in Fig. 5a. The electric field intensity and the specific conductivity are given in *XY* cross-section (Z=2 mm plane) of the three-dimensional finite-elements model. First part of electric field intensity and electrical conductivity is presented on Fig. 5a1-3 and b1-3, while second part of electrode commutation sequence is presented on Fig. 5a4 and b4.

2.3. Electrochemotherapy

In vivo electrochemotherapy experiment was performed at the Department of Experimental Oncology, Institute of Oncology, Ljubljana, Slovenia in accordance with ethical provisions for research on animals.

In experiment subcutaneous SA-1 fibrosarcoma syngeneic to A/J mice was initiated by injection of 5×10^5 cells into the left flank of the animal. SA-1 cell suspension was cultivated in Eagle Minimal Essential Media with 10% of Foetal Calf Serum (MEM, FCS; Sigma, ZDA). Ten days after subcutaneous injection of cells, the tumours were large enough (volume≈300 mm^3, diameter≈12 mm) for electroporation with multiple needle electrodes.

During the electrochemotherapy animals were anaesthetized with ketamin and rompun (2 μl of ketamin+8 μl of 0.9% physiological solution and 0.5 μl of rompun+9.5 μl of 0.9% physiological solution per gram of mouse). Animals were divided into four experimental groups: control, chemotherapy (CT), electric pulses (EP) and electrochemotherapy (ECT). In each experimental group 7 mice were treated independently. In all experimental groups multiple needle electrodes were inserted into the tumour. Animals in CT and ECT experimental

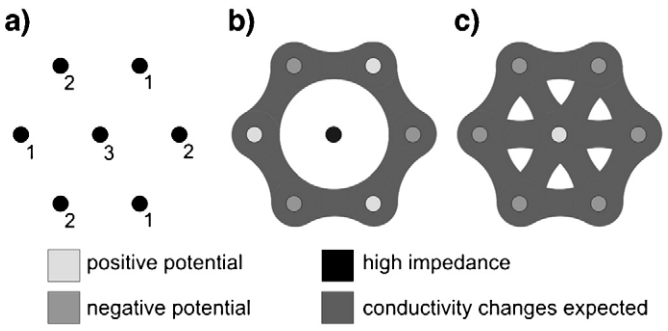

Fig. 4. Electrode commutation sequence of two parts for honeycomb arrangement of electrodes. Position of the electrodes and its numeration (a). States of the electrodes in the first part of electrode commutation sequence (b). States of the electrodes in the second part of electrode commutation sequence (c).

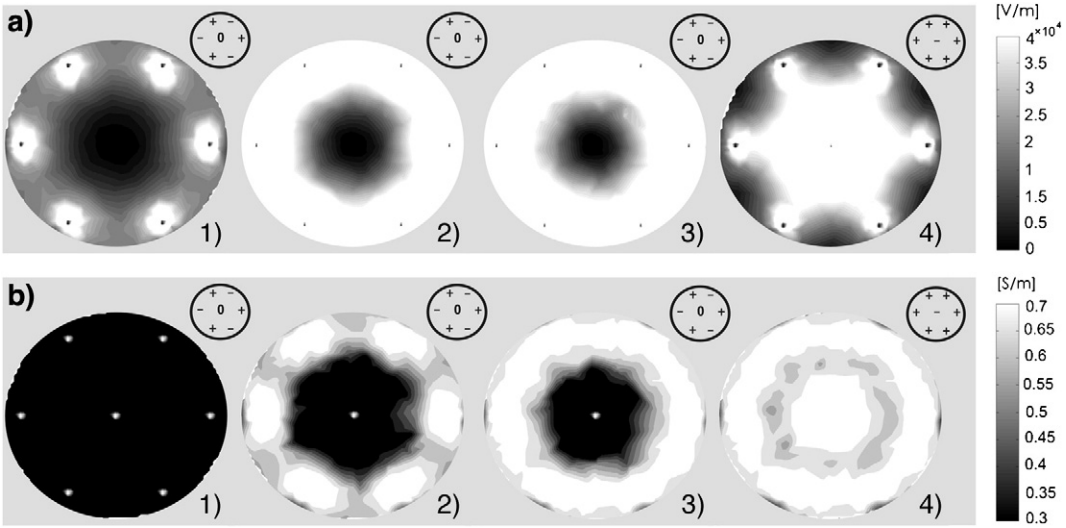

Fig. 5. Electric field intensity [V/m] (a) and electrical conductivity [S/m] (b): the sequence analysis was not applied (1), the first static model in the first part (2), the final static model in the first part (3) and the final static model in the second part of the electrode commutation sequence (4).

groups were injected intravenously with 100 μg of bleomycin. Tumours in experimental groups EP and ECT were exposed to electric pulses (2 intervals×8 square pulses, duration 100 μs, amplitude 530 V and pulse repetition frequency of 100 Hz). Pulses in the ECT group were delivered 3–4 min after the bleomycin injection.

Each day after the treatment on day 0 cranial/caudal, dorsal/ventral and medial/lateral diameters of the tumours were measured. Volumes of tumours were then modelled and calculated as spheroids. Results are given in form of scatter graphs (SigmaPlot 9.0, Systat, USA), where each point represents the mean volume of tumours in each experimental group and the error bars indicate the standard error of the mean (Fig. 6).

3. Results

Electric field intensity (Fig. 5a) in tumour tissue and consecutive tumour tissue conductivity changes (Fig. 5b) were calculated for suggested electrode commutation sequence (Fig. 4) by means of finite-elements method. In Fig. 5a1 and b1 sequence analysis was not yet applied. Fig. 5a2 and b2 represents first static model of sequence analysis. And Fig. 5a3,4 and b3,4 represents final static model of sequence analysis. In the first part of electrode commutation sequence (all outer electrodes are activated and neighbouring needles are of the opposite polarity, while the middle electrode is in high impedance state; Fig. 4b) when sequence analysis was not yet applied electric field intensity was strong only around outer electrodes (Fig. 5a1). When sequence analysis was applied electric field intensity (Fig. 5a2) and tumour tissue conductivity (Fig. 5b2) quickly iterated to its final value, which was obtained with insignificant numerical error in only four steps (Fig. 5a3 and b3). Strong electric field intensity around outer electrodes distributed around all outer part of tumour tissue (Fig. 5a3). We can see that after the first part of electrode commutation sequence all outer part of tumour tissue changed its conductivity which can also be considered as it was electroporated while the tumour tissue around the middle electrode remained unchanged i.e. not porated (Fig. 5b3). In the second part of electrode commutation sequence (all outer electrodes are positive and the inner electrode is negative; Fig. 4c) electric field intensity was particularly strong around inner electrode (Fig. 5a4) also because outer tumour tissue was already electroporated.

After completed electrode commutation sequence tumour tissue was thus well electroporated (Fig. 5b4), which was demonstrated also by electrochemotherapy effectiveness (Fig. 6). The suggested electrode

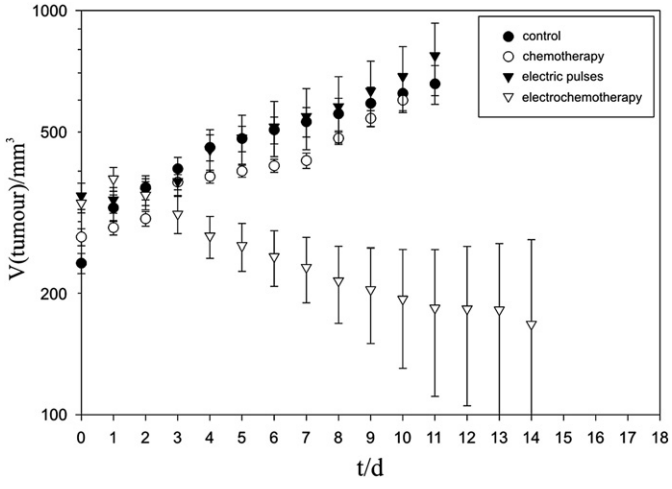

Fig. 6. Tumour growth after electrochemotherapy (ECT) on day 0 and three standard control groups for ECT: control, chemotherapy (CT) and electric pulses (EP). Each symbol represents an average tumour volume and standard error in specific experimental group.

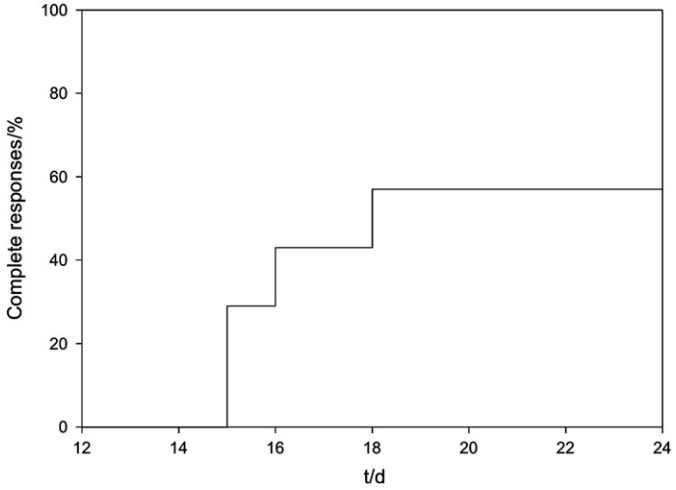

Fig. 7. Complete responses of tumour treatment on A/J mice after electrochemotherapy (ECT) on day 0. Tumours were observed for 3 months after day 0.

commutation sequence for honeycomb arrangement of seven-needle electrodes was evaluated in *in vivo* electrochemotherapy (ECT) experiment on larger tumours. Three standard experimental groups for evaluating ECT were used: control, chemotherapy (CT) and electric pulses (EP). Tumours in these experimental groups were not retarded in growth and animals were sacrificed after 10 days due to large tumours. In ECT group where tumours were subjected to the electrochemotherapy on day 0, tumours were however significantly reduced in volume and delayed in growth. In addition, 57% (4/7) complete responses were obtained (Fig. 7).

4. Discussion and conclusion

The aim of our study was to develop and test an effective *in vivo* electrochemotherapy on larger tumours by means of multiple needle electrodes. We thus developed and tested an electrode commutation circuit, which commutates the usual single output electroporation signal between the seven-needle electrodes used in our study. We suggested and tested experimentally by performing electrochemotherapy an effective electrode commutation sequence for tissue electroporation. Results of electrochemotherapy show a significant reduction in tumour volume, delay in growth and 57% complete responses (Fig. 6 and 7). We also calculated corresponding electric field distribution in tumour tissue taking into account an increase of tissue conductivity due to electroporation. Results of calculations show that large volume of tumour tissue is successfully electroporated by using suggested electrode commutation sequence (Fig. 5b4).

Electroporation is a dynamic process, meaning that tissue properties are changing during the constant voltage pulse from 0.3 to 0.7 S/m [22,23]. Electric field intensity below the reversible threshold value does not permeabilize the cell membranes and therefore no changes in conductivity are expected. When the electric field intensity exceeds reversible threshold cell membrane is permeabilized and tissue conductivity increases. The membrane permeabilization is reversible for electric field intensities below irreversible threshold.

The main purpose of the modelling was to foresee electroporated tissue in treated tumours. We thus had to take into account the influence of electric field intensity on tumour tissue conductivity and that changes in tumour tissue conductivity retroact on electric field distribution. Therefore sequence analysis was used to take into account such retroactivity. Results obtained with this application describe a sequence of static models, where each of them describes the process at one discrete interval. Each discrete interval relates to a real yet undetermined time interval. If we compare first (Fig. 5b2) and the last (Fig. 5b3) result of sequence analysis iteration we can see such retroactivity in results of tumour tissue conductivity. These results demonstrate that larger volume of electroporated tumour tissue is expected if sequence analysis is applied. Such expectations are in agreement with electrochemotherapy results (Figs. 6 and 7).

In *in vivo* electrochemotherapy experiment multiple needle electrodes in honeycomb arrangement were used, because such arrangement of electrodes is preferable in electrochemotherapy as better coverage of tumour tissue with electric field distribution can be achieved with single amplitude of electric pulses. For such electrodes an effective and short pulse electrode commutation sequence for electroporation is required. In our study we used a combination of two parts, which are presented on Fig. 4. Such sequence of voltage pulses application is quick, because it is delivered in only two parts and therefore suitable for clinical use where patients are subjected to muscle contraction and painful sensations. It is also effective as the conductivity changes expected due to tissue permeabilization are favouring electric field distribution through all the area between the electrodes.

Embedded electrode commutator, which was developed and used in our study, has been proven to be effective and was therefore built in the Cliniporator device [24]. Moreover, such electrode commutator is relatively easy to construct due to simple design and can be used for any other single output electroporator. On the basis of our experimental and numerical results we can conclude that suggested electrode commutation sequence for honeycomb arrangement of electrodes can be efficiently used in electrochemotherapy. Given output amplitude allows for treatment of larger tumours using multiple needle electrodes without repositioning of electrodes.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bioelechem.2008.03.001.

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