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Electric field modulation in tissue electroporation with electrolytic and non-electrolytic additives

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Abstract

Electroporation, cell membrane permeabilization with short electrical field pulses, is used in tissue for *in vivo* gene therapy, drug therapy and minimally invasive tissue ablation. For the electroporation to be successful, the electrical field that develops during the application of the pulses needs to be precisely controlled. In this study we investigate the use of electrolytic and non-electrolytic gels to generate the precise electrical fields required for controlled electroporation, in heterogeneous and irregular tissues, *in vivo*. Finite element computer simulations are used to illustrate various applications, such as the treatment of irregularly shaped organs and interior cavities. The feasibility of the concept is demonstrated experimentally *in vivo* with a rat liver subjected to irreversible electroporation.

Keywords: Electroporation; Electropermeabilization; Electrolytic gel; Gene therapy; Irreversible electroporation

1. Introduction

Electroporation, or electropermeabilization, is the phenomenon in which cell membrane permeability to ions and macromolecules is increased by exposing the cell to short (microsecond to millisecond) high electric field pulses [1]. Reversible electroporation of living tissues is the basis for different therapeutic maneuvers on clinical use or under study [2]: *in vivo* introduction of genes into cells (electrogenetherapy) [3–5], introduction of anti-cancer drugs into undesirable cells (electro-chemotherapy) [6] and introduction of photosensitizers into tumor cells for photodynamic therapy [7]. Irreversible electroporation has also found a use in tissues as a minimally invasive surgical procedure to ablate undesirable tissue without the use of adjuvant agents [8–10].

Electroporation is a dynamic phenomenon that depends on the local transmembrane voltage at each cell membrane point. It is generally accepted that for a given pulse duration and shape, a specific transmembrane voltage threshold exists for the manifestation of the electroporation phenomenon (from 0.5 V to 1 V). This leads to the definition of an electric field magnitude threshold for electroporation (E_{th}). That is, only the cells within areas where $E \geq E_{th}$ are electroporated. If a second threshold (E_{th_irr}) is reached or surpassed, electroporation will compromise the viability of the cells, i.e., irreversible electroporation.

It is obvious, from the above, that precise control over the electric field that develops in tissues is important for electroporation therapies [11–14]. For instance, in reversible electroporation it is desirable to generate a homogeneous electric field ($E_{th} \leq E < E_{th\_irr}$) in the region of interest and a null electric field in the regions not to be treated. Currently, optimization of the electric field distribution during electroporation is done through design of optimal electrode setups [15]. However, there are situations in which an electrode setup alone is not sufficient for obtaining an optimal electrical field, particularly in situations such as the electroporation of irregularly shaped tissues or when the protection of specific tissue regions is required.

Here we propose and investigate the use of additives for modulating the electric properties of the treated tissues or for modifying the geometry of tissues or electrodes as a means of optimizing the electric field during tissue electroporation. As an

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example of additives, in this paper we will explore the use of fluids with various conductivities and, more specifically, the use of gels with various ionic contents. We found gels particularly interesting because they can behave as solids but they can also be injected easily with a syringe.

There are numerous uses of this concept, some of which are listed below. As reported in the following subsection, it should be emphasized that the use of additives for modulating the electric field is not new. What we show in this paper is the wide range of additional possible applications of the concept. We will discuss the different applications in the introduction section and then illustrate the concepts with analysis and experimentation in the following sections.

The layout of the paper is as follows. Within the introduction Section 1, Sections 1.1–1.4 introduce the basic concepts of how additives can be employed to modulate the electric field in different electroporation generic cases. Section 1.5 explains why and how gels can be used for such a purpose. Section 2, "methods", provides details of how computer simulations have been carried out to illustrate the cases presented in Section 1, furthermore, it explains the procedure of an experiment in which a rat liver lobe was irreversibly electroporated using an electrolytic gel to show the feasibility of the strategy.Section 3, "results and discussion", shows the results from computer simulations of examples related to the typical cases introduced in Section 1. Then, it also presents the results from the *in vivo* experiment and relates them to computer simulation results.

1.1. Conditioning of tissue electrical properties or of electrode–tissue interfaces

When in electroporation a current is forced to flow across different tissue layers, those with higher resistivity will be subjected to higher electric fields. This implies that some tissue layers will be more prone to electroporation than others. Although, this is not necessarily an inconvenience, it could imply that in order to reversibly electroporate some tissues it would be necessary to irreversibly electroporate others, if not to burn them because of the Joule effect [16]. Moreover, the voltage drop at those higher resistivity layers will be significant and, in most cases, uncontrollable. Thus, in these cases it will be difficult to assess the required external voltage in order to have the sufficient electric field at the region of interest.

Furthermore, the electrode–tissue interface impedance is also inconvenient in the same sense. At the electrode surface, electron exchange reactions occur that transform electronic transport (electrode metal) into ionic transport (tissue). Such transformation also implies a resistance, and a resulting voltage drop, that will depend on different factors such as the availability of ions and their mobility.

A case that combines both phenomena is the skin-fold electroporation technique in which skin is folded and electroporated with parallel plates on opposite sides of the fold [17]. In most cases, skin viable tissue layers are the objective of the treatment whereas the top layer, the stratum corneum, represents an impediment to the treatment because of its high resistivity. Moreover, since the electrode–tissue interface in this case is rather dry, the availability and mobility of ions is poor and the related interface resistance is quite high. In fact, this is not only a problem for electroporation but also for different bioelectric applications involving electrodes such as external defibrillation. In these other cases, electrolytic gels and pastes have been used for decades [18]. Therefore, it is not surprising that gels have also been adopted by researches in the electroporation field in order to improve the "contact" between the electrodes and the tissues [11,13]. These compounds improve both the electrode–tissue interface impedance and the skin top layer conductivity by supplying water and ions, even in some cases they include abrasives to help to reduce the resistance of the stratum corneum layer.

Hence, conductive gels are known in the electroporation field. However, we will show that in electroporation there are many additional valuable applications of electrolytic and non-electrolytic additives and gels, in addition to improving the "contact" impedance.

1.2. Insulation of tissue regions

During electroporation, it is often important to ensure that certain regions of tissue are not affected by the applied electric field. One possible method to achieve this is by isolating the treated region from that to be protected with non-conductive gels, i.e. without free ions. However, it must be taken into account that: 1) once the gel is in contact with tissues, biological ions will begin to diffuse inwards and, consequently, its conductivity will increase and its behavior as insulator will be compromised after a period of time; and 2) the gel must be perfectly deposited as a continuous layer, otherwise, any cleft or hole will lead to conductive paths. Therefore, we believe that it is better to consider the use of non-conductive gels as "injectable spacers" rather than as insulation films. That is, we propose to use them to physically separate the region to be electroporated from those to be protected. Fig. 1 illustrates a possible application of such strategy; the top layer, to be treated by means of irreversible electroporation, could represent a skin melanoma whereas the bottom region could represent any hypodermic tissue, such as muscle, to be protected. In this case, the gel would be injected hypodermically through a syringe before the application of the needle electrodes

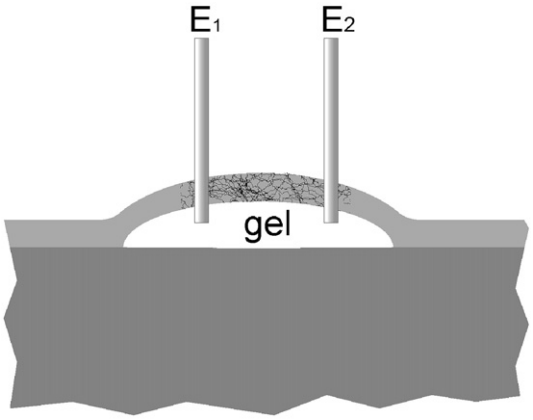

Fig. 1. Non-conducive gels can be used to protect specific tissues from electroporation.

(E_1 and E_2). Section 3.1.2 shows the simulation results of a structure resembling this case.

1.3. Electric field homogenization in irregularly shaped tissues

Two parallel plate electrodes produce an almost homogeneous electric field distribution when a homogeneous tissue slab is placed in between them, as in the case of the skin-fold technique. However, as we will show inSection 3.1.2 (Fig. 4c), plate electrodes do not produce homogeneous electric fields when the tissue part to be treated has an irregular shape. A possible solution to this problem, in the spirit of using additives to modulate the electrical properties of tissue, is to fill the space between plate electrodes with a gel whose conductivity is equal or similar to that of the tissue to be electroporated ("matched conductivity gel"). By doing this, the material between the plates will become homogeneous in electrical terms and the generated electric field distribution will also be homogeneous. Computer simulations show that perfect matching between gel and tissue conductivities is not required in order to obtain very significant improvements.

Section 3.1.2 shows the simulation results of a hypothetical case related to the above. That example could represent an irregularly shaped hard tumor that needs to be reversibly electroporated through external plate electrodes.

Section 3.2 shows an *in vivo* experimental verification of the concept. The edge of a rat liver lobe was irreversibly electroporated between two plate electrodes. In order to homogenize the electric field, a "matched conductivity gel" was employed to fill the space between the electrodes and the liver surface.

Besides geometrical irregularities on the surface of the tissue, large blood vessels could also have a significant impact on electric field distribution. One would expect that due to their higher conductivity they may cause important field heterogeneities. In fact, we have observed treatment heterogeneities when applying electroporation through needle electrodes (not reported here) that we attribute to such phenomenon. A possible solution is the perfusion of blood vessels with fluids of similar conductivity to that of the parenchyma to be treated.Section 3.1.3 shows the simulation results of a hypothetical case in which reversible electroporation of a region that contains a blood vessel in its vicinity is performed. In this case, only a thin strip of tissue is not electroporated because of resulting field distribution heterogeneity. However, in the case of tumor ablation through electro-chemotherapy this could have dramatic consequences, particularly taking into account that the surviving tumor cells would be close to a blood vessel. Another simulation result in Section 3.1.3. shows that the effect of replacing the blood with a tissue matched conductivity fluid has a positive impact.

1.4. Implementation of injectable electrodes

High conductivity gels are good conductors and, therefore, they can also serve as electrodes. This implies that it is possible to implement electrodes that are soft, injectable, moldable and biodegradable, among other possible interesting features. Here we describe two possible applications of such injectable electrodes:

1.4.1. Shielding of tissue regions

When voltage is applied between two electrodes, current flows through the path of least resistance, which, in most cases, coincides with the shortest path. Hence, a possible way to guarantee that a specific tissue region will be not electroporated is to place it behind the electrodes, that is, outside of the region sandwiched by the electrode pair. In some cases it will be possible to actually displace the tissues or to use spacers for separation (Section 1.2). In other cases, the strategy depicted in Fig. 2a could be employed. That is, to implement an embedded electrode in such a way that the tissue region to be protected (bottom layer in Fig. 2a) lies outside of the region between both electrodes.

The example depicted in Fig. 2a could represent a case of skin electroporation in which the muscle and other deeper structures need to be protected. The process would start by injecting the gel subcutaneously through a syringe. Then, the same injection needle with an electrical insulation on the shaft or a wire threaded

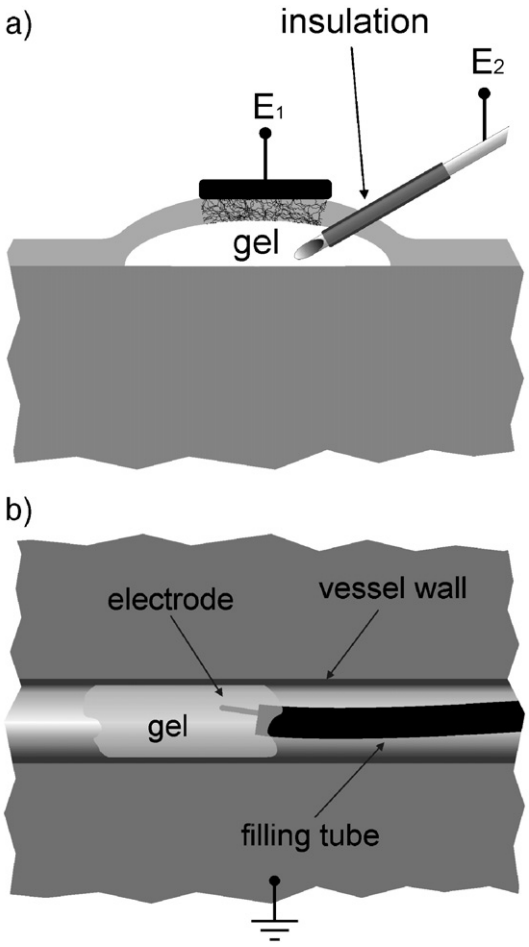

Fig. 2. Possible uses of injectable electrodes. (a) Protection of specific tissue regions. Gel electrode is injected beneath the top layer. When voltage pulse is applied, only the region comprised between the gel and top electrode is electroporated. (b) Electroporation of hollow structures. Gel electrode is injected through a catheter that is also used to connect it to the pulse generator. The opposite electrode can be placed on the surface of the body.

through the skin and gel would be used for the electrical contact between the gel region (injected electrode) and the pulse generator terminal. In this way, roughly only the skin between the gel and the top electrode would be electroporated. An interesting feature of the proposed method is that the injectable electrodes could adapt to the morphology of the region to be treated.

Section 3.1.4 contains simulation results of a hypothetical case which shows how injected electrodes can be employed to protect specific tissue regions. In that case, however, the objective is to reversibly electroporate the inner tissue without damaging the outer layers.

1.4.2. Method to electroporate hollow structures

Electroporation of blood vessel tissues is possible through intraluminal catheters which take advantage of the conductivity provided by blood [19]. However, in other cases, such as the gastrointestinal or urinary tracts, there is no natural electrical contact media between the intraluminal electrode and the organ walls. Flexible electrodes designed to make direct contact with walls to be treated have been proposed for these cases [20]. Here we suggest that the additives investigated in this study, such as conductive gels or pastes could be a much simpler and yet effective solution. For instance, as shown in Fig. 2b, a catheter could be used to inject the conductive gel into a vessel which when brought in contact with a wire further connected to the power supply could serve as an electroporation electrode. An advantage of this strategy is that gel preparation could also contain the therapeutic agent and there would be no need to entrap it with balloons or other means in the region to be treated as it is the case in intraluminal catheter for electroporation [19].

In the example in Fig. 2b one of the electrodes is the injected gel and the other would be a large electrode on the surface of the body. In this way, highest electric fields will be produced around the gel, particularly if the vessel wall has lower conductivity than surrounding parenchyma, as it will happen in most cases. Thus, only an annular region surrounding the gel will be electroporated. Such structure is simulated in Section 3.1.5.

1.5. Ionic gels

Gels are colloidal dispersions in which the dispersion medium is a liquid and the continuous medium is a solid, generally a network of polymeric chains. In the specific case that water is the liquid medium, gels are also called hydrogels. An interesting property of most gels is thixotropy, that is, they become more fluid when mechanically disturbed. Thus, whereas in steady state gels can behave like a soft solid or a high viscoity fluid, they can be injected easily through small gauge needles thanks to the effect of shear forces. Hydrogels are used extensively for various medical applications such as breast implants, wound dressings materials, drug delivery systems, electrodes and contact lenses. Features such as biocompatibility, biodegradability and temperature and chemical sensitivity can be achieved.

A straightforward method to generate a liquid or gel with a desired electric conductivity is by controlling the content of free ions. Presumably, hypoionic solutions will have no significant effect on living tissues if they are applied for short periods and if they are balanced with non-ionic species to achieve isotonicity. On the other hand, high conductivity gels will almost certainly imply hypertonicity. Hence, some damage to the tissue due to osmotic unbalance (cytotoxicity caused by cell dehydration) might be expected. In fact, hypertonic gels have been proposed as an ablation method [21]. Nevertheless, although very highly hypertonic gels (23.4% NaCl) have been tried, the observed lesions remained small. Quite fortunately, according to the theoretical results presented here, it seems that it will not be necessary to employ concentrations above 15% NaCl ($\sigma \approx$ 240 mS/cm). Thus, taking into account that the presence of the hypertonic gels will only be required for a short period, we do not foresee problems regarding the biocompatibility of the materials.

2. Methods

2.1. Electric field distribution computed by the finite element method

In this study we use mathematical analysis to explore and illustrate the various applications of the electrolytic and non-electrolytic gels to control electroporation. To this end we employ the *finite element method* (FEM) to compute the electric field distribution under the assumption of constant conductivities and static currents and fields. This methodology has been used by previous researchers in the field [22–24] and its validity has been proven [10,12].

The key idea of the FEM is the decomposition of an arbitrary geometry into small simple elements in which it is possible to solve the differential equations related to the phenomena under study. Given the appropriate boundary conditions, the solutions are then assembled and an approximate solution for the complete geometry is provided. In our case, the solved equation for each element is the Poisson's equation:

$$-\nabla \cdot (\sigma \nabla V - J^{e}) = Q_{j} \tag{1}$$

where σ is the conductivity, V is the voltage, J^{e} is a vector denoting the externally generated current density and Q_{j} indicates the current generated in the element (null in all the cases presented here).

The specific FEM tool used here was COMSOL Multiphysics 3.2 (www.comsol.com) and the mode chosen for the simulations was "3D conductive media DC". The boundary conditions were all insulating on the external surfaces. Unstructured meshes of tetrahedral elements were automatically generated by the FEM tool.

The geometry of the analyzed cases and other details relevant to the simulations are discussed in next sections. Unless otherwise specified, the simulations have been performed assuming that E_{th} = 500 V/cm (reversible electroporation threshold), E_{th_irr} = 1000 V/cm (irreversible electroporation threshold), and the conductivity of the tissue (σ) is 1 mS/cm. In the resulting graphs black color indicates $E < 500$ V/cm (no effect); grey color 500 V/cm $\leq E <$ 1000 V/cm (reversible electroporation); and white $E \geq$ 1000 V/cm (irreversible electroporation).

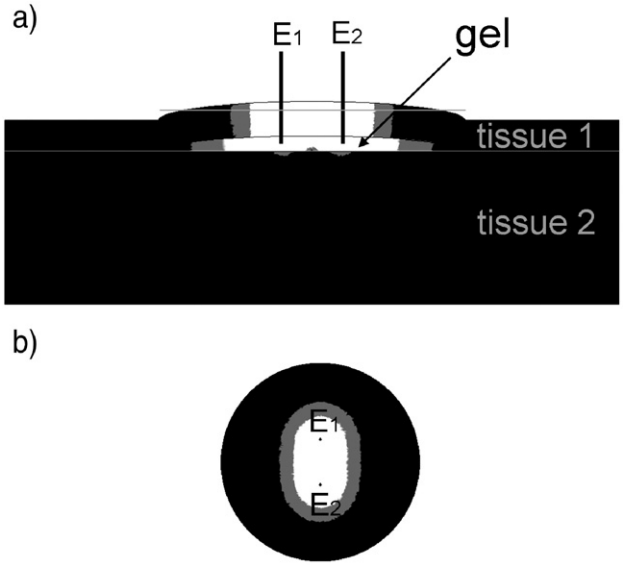

Fig. 3. Simulation result of tissue region protection by means of non-conductive gel used as a spacer (tissue 1 conductivity = tissue 2 conductivity = 1 mS/cm, gel conductivity = 0.1 mS/cm; gel region diameter = 10 mm, gel region height = 0.5 mm; tissue 1 thickness = 1 mm; electrode diameter = 0.1 mm, electrode separation distance = 2 mm). (a) Vertical plane that comprises both electrode axes. (b) Horizontal plane at the height denoted by gray line in above figure (~ 1/4 of tissue 1 thickness at region of interest). Black color indicates $E < 500$ V/cm (no effect); gray color 500 V/cm $\leq E < 1000$ V/cm (reversible electroporation); and white $E \geq 1000$ V/cm (irreversible electroporation).

2.2. In vivo proof of concept

To demonstrate the feasibility of using gels for electroporation, we carried out, in addition to the mathematical analysis, an experiment in which the edge of a rat liver lobe was irreversibly electroporated between two plate electrodes.

From previous experimental studies [10], we know that irreversibly electroporated regions in rat liver show within 30 minutes entrapping of erythrocytes that is observable both macroscopically (darkening due to blood congestion) and microscopically. Thus, this phenomenon can be employed to assess the interface between reversible and irreversible electroporated regions and thereby distribution of electric fields during electroporation.

2.2.1. Experimental procedure

A male Sprague–Dawley rat (350 g) was obtained from Charles River Labs through the Office of Laboratory Animal Care at the University of California, Berkeley. It received humane care from a properly trained professional in compliance with both the Principals of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals, prepared and formulated by the Institute of Laboratory Animal Resources and published by the U.S. National Institutes of Health (NIH).

The experiment started with anesthetization of the animal via intraperitoneal injection of Nembutal solution (50 mg/ml sodium pentobarbital, Abbott Labs, North Chicago, IL) for a

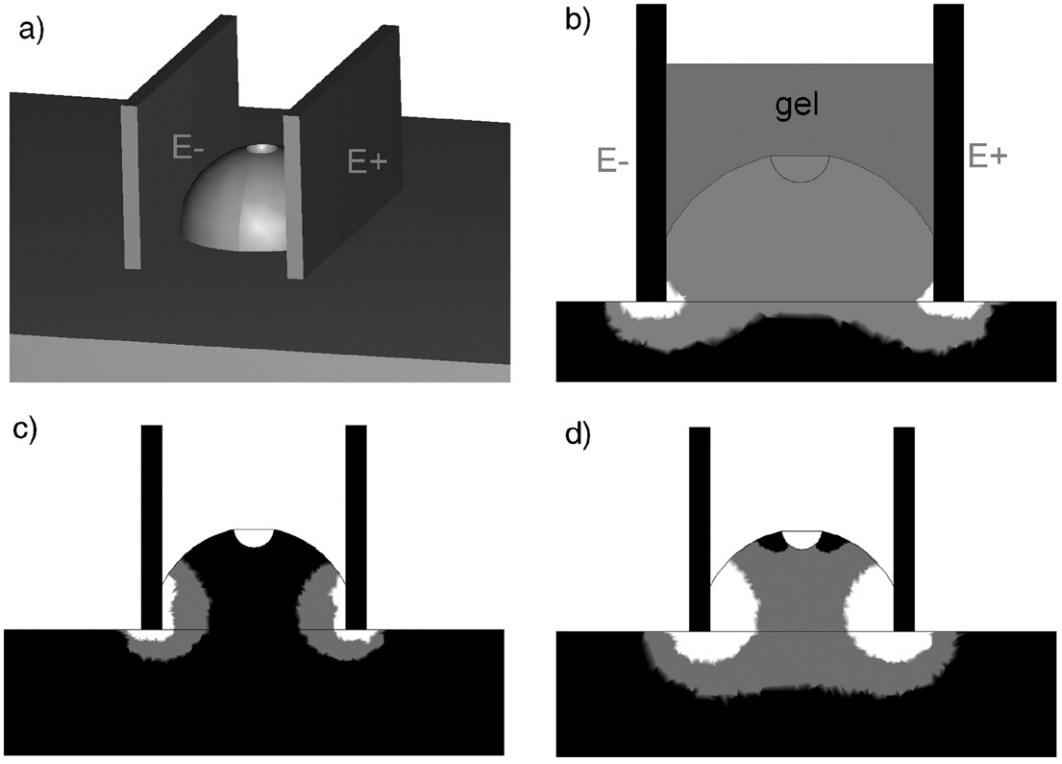

Fig. 4. Simulation of the electroporation of a semi-spherical tumor (diameter = 1 cm) at 550 V. (a) Model employed in the simulation. (b) Resulting electric field magnitude from the simulation when gel is present (vertical plane across the center of the electrodes). (c) Simulated electric field magnitude when matched conductivity gel is not employed (vertical plane across the center of the electrodes). (d) Same as Fig. 4c but now voltage between electrodes is 1100 V.

total of 100 mg sodium pentobarbital per kg of rat. 30 min later, the liver was exposed via midline incision.

After exposing the rat liver, a liver lobe was placed between two flat circular electrodes separated at a distance of 5 mm. In order to emulate a geometrical irregularity, the liver lobe edge was inserted partially between the two electrodes. The placement of the electrodes in relation to the liver resembled the illustration in Fig. 8, which at the same time is the model for the FEM simulation of the case. Then, the void space was filled with the matched conductivity gel and the electroporation pulse sequence (8 pulses of 750 V with a duration of 100 μs and a period of 100 ms) was applied by means of a commercial pulse generator (ECM 830, Harvard Apparatus; Holliston, MA).

Two hours and a half after the electroporation sequence, the animal was euthanized and liver samples were prepared for histological analysis.

2.2.2. Histology

To fix the liver at its current state for microscopic viewing, we flushed the vasculature with physiological saline for ten minutes at a hydrostatic pressure of 80 mmHg from an elevated IV drip. This was accomplished by injecting the fluid into the left ventricle and letting it exit from a cut made in the right atrium. Immediately following saline perfusion, a 5% formaldehyde fixative was perfused in the same way for ten minutes. The treated liver lobe was then removed and stored in the same formaldehyde solution. Hematoxylin–eosin staining was then performed on cross-sections through the center of the treated region to study the effects of electroporation.

2.2.3. Gel preparation

We prepared a saline gel from a 0.045% NaCl solution, which is 20 times less concentrated than the standard physiological solution (0.9% NaCl). Such electrolyte content should produce an

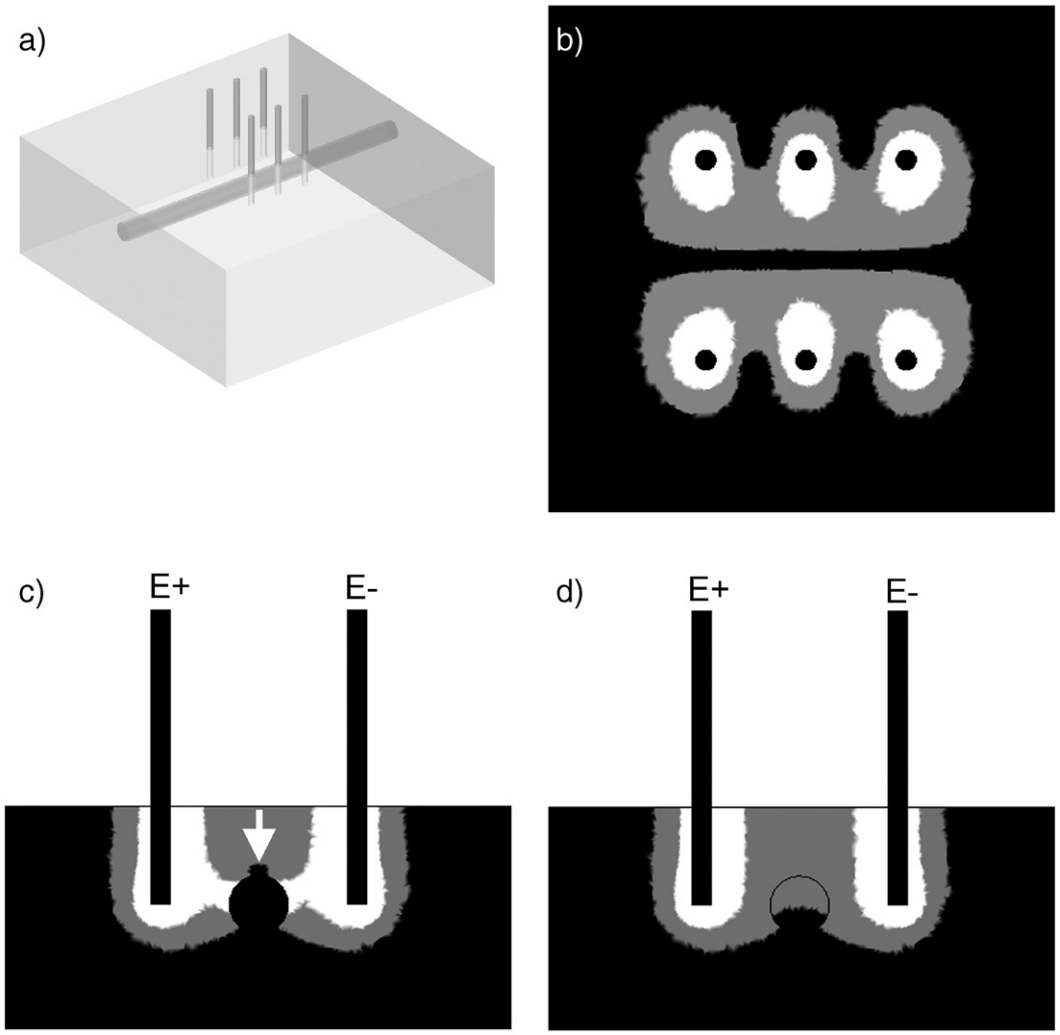

Fig. 5. Simulation of the reversible electroporation of a square region that contains a blood vessel (diameter = 3 mm) on its bottom boundary. (a) Model employed in the simulation, each electrode (E+ and E−) is constituted of an array of three needles (diameter = 1 mm, separation = 5 mm, penetration depth = 5 mm); the separation distance between both arrays is 10 mm; blood vessel conductivity = 10 mS/cm and tissue conductivity = 1 mS/cm; applied voltage = 1000 V. (b) Horizontal section of the simulated field magnitude at the top interface between the blood vessel and the parenchyma. (c) Vertical section, across the array centers, of the simulation results; the white arrow indicates the region the on top of the blood vessel that is not electroporated at all. (d) The same result when blood vessel conductivity is changed from 10 mS/cm to 1.5 mS/cm.

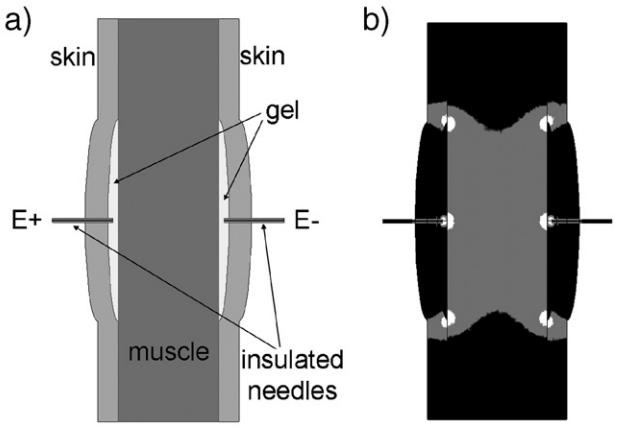

Fig. 6. Simulation of muscle (thickness = 5 mm) electroporation at 700 V through injected semi-ellipsoidal gel regions connected to metallic needles with insulation on their shaft (E+ and E−). (a) Model employed in the simulation; gel conductivity = 200 mS/cm, muscle conductivity = 1 mS/cm, skin conductivity = 0.1 mS/cm. (b) Resulting electric field magnitude from the simulation. The number of elements of the mesh is 196,534.

electrical conductivity of around 0.7 mS/cm. Reported liver conductivities maybe a little bit higher (~ 1 mS/cm), however, as seen in the simulations, this difference should not produce significant effects. The steps to produce the gel were: 1) add 0.8 g of raw agar to 100 ml of a 0.045% NaCl solution; 2) dissolve agar in the saline solution at boiling point; 3) cool the solution until solidification and 4) stir until gel formation.

3. Results and discussion

3.1. Simulation of typical applications

The goal of this section is to illustrate the concepts brought in the introduction with typical examples.

3.1.1. Tissue insulation

Here we simulate the case discussed in Fig. 1 (Section 1.2). That is, non-conductive gel is injected underneath the top layer of tissue in order to physically separate it from bottom tissue, which needs to be protected. This particular case could represent a skin melanoma that needs to be removed by irreversible electroporation.

The model used for the simulation consists of five components: 1) a square prism (20 mm × 20 mm × 5 mm) with conductivity = 1 mS/cm that models bottom tissue (tissue 2); 2) a half ellipsoid volume (10 mm × 10 mm × 0.5 mm) on top of the prism with conductivity 0.1 mS/cm for the insulating gel; 3) a mantle (thickness = 1 mm) with conductivity = 1 mS/cm on top of both components that models top tissue (tissue 1); and 4) and 5) two cylinders (diameter = 0.1 mm, length = 5 mm) with high conductivity (1000 S/cm) representing the electrodes that penetrate the top mantle. The separation distance between the electrodes is 2 mm and the applied difference voltage is 1000 V. The number of elements of the mesh is 102,614.

Note that we have chosen a conductivity of 0.1 mS/cm for the insulating gel, instead of the ideal 0 mS/cm, to take account of the fact that impurities in the gel and ionic diffusion from tissue after implantation will make it not a perfect insulator.

The result of the simulation (Fig. 3) shows that, although the top tissue is irreversibly electroporated throughout the entire area surrounding the electrodes, damage to the bottom layer is minimal and only two single spots experience reversible electroporation. It is interesting to note that the simulated gel is not completely non-conductive, in fact, its conductivity is only one order of magnitude lower that the conductivity of the tissues.

3.1.2. Electric field homogenization in irregularly shaped tissues

The example presented here is relevant to the application discussed in Section 1.3. That is, the homogenization of the electric field in irregularly shaped tissues by means of matched conductivity additives.

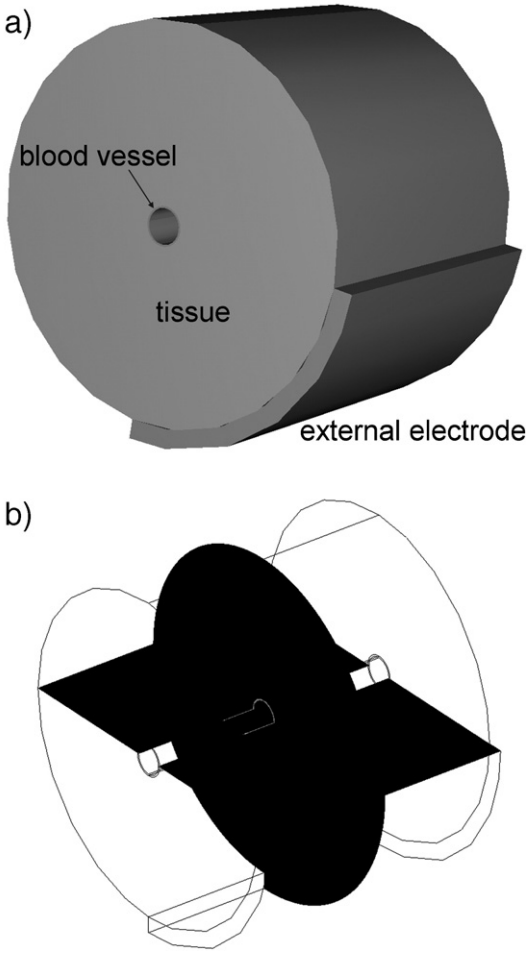

Fig. 7. Simulation of empty blood vessel (outer diameter = 10 mm, inner diameter = 9 mm) electroporation at 200 V through an injected cylindrical gel region and an external metallic electrode. (a) Model employed in the simulation; gel cylinder is at the centre of the geometry, within the vessel; gel conductivity = 200 mS/cm, tissue conductivity = 1 mS/cm, vessel wall conductivity = 0.25 mS/cm. (b) Resulting electric field magnitude from the simulation, two transverse cross-sections at the center of the geometry are shown. Although it is difficult to appreciate, it can be observed that only the vessel walls at the location of the gel cylinder have been reversibly electroporated.

The structure depicted in Fig. 4a could correspond to the case of an irregularly shaped hard tumor electroporated through plate electrodes. The model consists of a semi-sphere (diameter = 10 mm) on top of a square prism (50 mm × 50 mm × 20 mm) that represents the tissue (conductivity = 1 mS/cm) and two plate electrodes (20 mm × 10 mm × 1 mm; conductivity = 1000 S/cm) on two opposite sides of the semi-sphere. An extra irregularity in the shape of a semi-spherical depression (diameter = 2 mm) has been included at the top of the tissue part. The number of elements of the mesh is 21,888. The voltage applied between electrodes is 550 V.

The simulation result of electroporation in the absence of a gel is shown in Fig. 4c. It is obvious that the electric field distribution is extremely heterogeneous. Furthermore, even in the case that very high voltages are employed (Fig. 4d), there are regions that are not electroporated. Of course, this is something not acceptable in cancer treatment and, maybe because of that, needle array electrodes are preferred for this kind of tumors rather than plate electrodes. On the other hand, when the addition of a matched conductivity gel is simulated (Fig. 4b) the results show that the electric field is much more homogeneous, even in this case in which the conductivity matching between tissue and gel conductivities is not perfect (matching error = 30%).

3.1.3. Electric field homogenization in tissues containing blood vessels

The simulation presented here is also related to Section 1.3. The current case represents the reversible electroporation of a region that contains a blood vessel in its vicinity (Fig. 5a). The electroporation is performed with two parallel arrays of needle electrodes that should produce a quite homogeneous field within the region between them.

The model used for the simulation consists of: 1) a rectangular prism (50 mm × 50 mm × 20 mm) that represents the tissue an has a of conductivity 1 mS/cm; 2) a cylinder that represents the blood vessel (conductivity = 10 mS/cm; diameter = 3 mm, length = 50 mm) that goes from one lateral side of the prism to the other at a depth of 5 mm; and 3) two electrode arrays placed in parallel at a distance of 10 mm between them. Each one of both arrays consists of three cylindrical rods (conductivity = 1000 S/cm; diameter = 1 mm, length = 15 mm, separation distance = 5 mm). The number of elements of the mesh is 68,571. The voltage applied between both arrays is 1000 V.

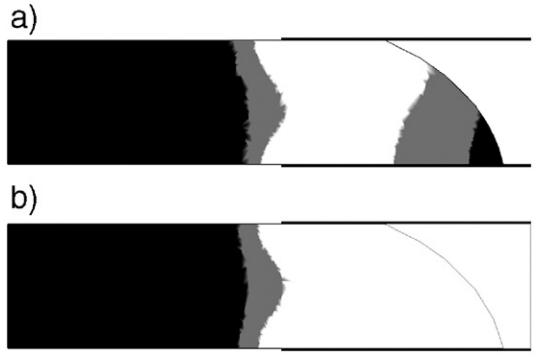

Fig. 9. Results from the simulation of the liver lobe electroporation (plane x–y, z=0). (a) Without gel; an irregular electroporation pattern is obtained, a significant proportion of the tip is not irreversible electroporated. (b) The presence of filling gel with conductivity = 0.8 mS/cm is simulated; the tip region between the electrodes is irreversibly electroporated.

Simulation results show that a thin strip of tissue is not electroporated because of resulting field distribution heterogeneity (Fig. 5b and c). On the other hand, when blood is replaced by a matched conductivity additive (vessel conductivity changes from 10 mS/cm to 1.5 mS/cm), the simulated field distribution (Fig. 5d) is much more homogeneous and the objective of completely electroporating the region between electrodes is achieved.

3.1.4. Subdermal electrodes

The example presented here is relevant to the application discussed in Section 1.4.1. That is, the use of injectable electrodes in order to protect specific tissue regions.

For the case shown in Fig. 6a, the objective is to reversibly electroporate the inner tissue (rectangular prism (20 mm × 20 mm × 5 mm); conductivity = 1 mS/cm) without damaging the outer layers (mantle with thickness of 1 mm; conductivity = 0.1 mS/cm). To perform such selective electroporation, we propose the injection of conductive gels at both sides of the region to be electroporated through needles with insulation on their shaft. The idea is that both regions will behave as parallel plate electrodes (gel regions are modeled here as half ellipsoid volumes

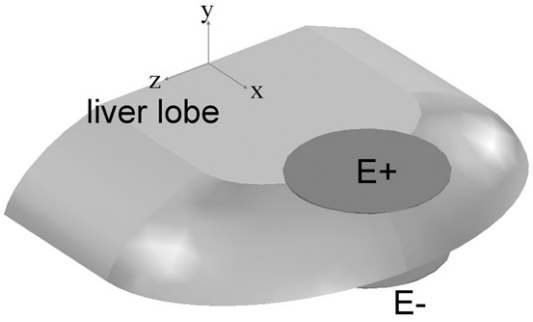

Fig. 8. Representation of the model employed to simulate the electroporation of the liver lobe tip (gel is not shown). The liver lobe tip is electroporated between the disk electrodes E+ and E− (diameter = 10 mm, conductivity = 1000 S/cm) separated at a distance of 5 mm. The applied voltage is 750 V and liver tissue conductivity is 1 mS/cm. The number of element of the mesh is 61,346.

Fig. 10. Picture of the electroporated rat liver lobe. It was cut and opened through the middle of the electroporated region. White dots are placed at the border between the treated tissue and the intact tissue.

(10 mm × 10 mm × 0.5 mm) with a conductivity of 200 mS/cm). Indeed, according to the simulation results (Fig. 6b) such behavior is obtained. Note, however, that enhancement of electric fields at gel region edges occurs and that causes in some damage to top tissues (skin).

3.1.5. Hollow structure electroporation

Here we show a simulation of the case presented in Fig. 2b. An empty vessel is filled in its central region with a high conductivity gel. Then, electroporation voltage is applied between this gel and an external large electrode (Fig. 7a). If the voltage is properly selected, significant results in terms of selectivity of electroporation can be achieved. This is illustrated by the result of the simulation (Fig. 7b). The figure shows that only the vessel wall in contact with the gel is electroporated.

The model (Fig. 7a) consists of a large cylinder (diameter = 100 mm, length = 80 mm) that stands for the tissue between the cavity and the external electrode (conductivity = 1000 S/cm); a cylindrical cavity (diameter = 9 mm) with infinite resistivity; a thin (thickness = 0.5 mm) wall between the cavity and the tissue with conductivity = 0.25 mS/ cm; and a cylinder (diameter = 9 mm, length = 20 mm) in the center of the geometry (not visible in Fig. 7a) that stands for the inner gel electrode and that has a conductivity of 200 mS/cm. The applied voltage between the internal electrode (inside the gel) and the external electrode is 200 V. The number of elements of the mesh is 56,364.

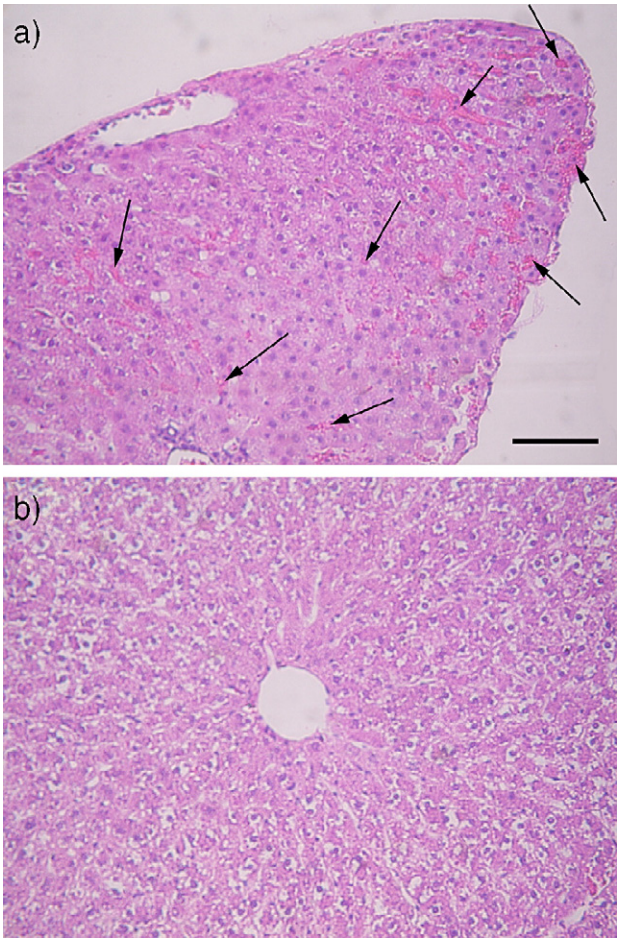

Fig. 11. Microscopic pictures of the electroporated liver lobe. (a) Tip of liver lobe; significant entrapping of erythrocytes (marked with arrows) denotes that irreversible electroporation has been produced. (b) Central vein area in an inner zone; no alteration can be observed. Bar indicates 100 μm, both pictures are at the same magnification.

3.2. In vivo proof of concept

The results from the simulation of the structure resembling the *in vivo* proof of concept model (Fig. 8) show that if no gel had been applied the very tip of the lobe would have not electroporated at all (Fig. 9a). On the other hand, when the gel is applied, the effect of a matched conductivity gel (matching error = 20%) is to cause complete irreversible electroporation of the whole lobe, including the tip (Fig. 9b). Macroscopic observation of the electroporated region (Fig. 10) before formaldehyde fixation agreed with the simulation result. Note that it is even possible to appreciate the concave shape of the irreversibly electroporated region that was predicted by the simulation. Microscopic observation (Fig. 11a) confirmed that entrapping of erythrocytes occurred through the whole lobe tip whereas it was not present in inner regions (Fig. 11b).

4. Conclusions

Here we proposed and explored the use of gels with various conductivities to optimize and control the distribution of the applied electric fields that result during electroporation tissue. We find of particular interest the potential use of matched conductivity gels in order to homogenize fields in irregularly shaped tissues. For instance, these gels could be employed in conjunction with plate electrodes for treating superficial hard tumors that cannot be treated by using the skin-fold technique. Because of the simplicity of the concept, we also think that the use of high conductivity gels as injectable electrodes and their use for the electroporation of hollow structures is of potential interest.

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