Finite Element Modeling of in Vivo Electroporation

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Introduction

As a simple definition, a mathematical model is a representation of chosen essential aspects of a real system (may it be a living, engineering or social system), described by a set of variables and a set of equations that establish relationships between the variables. The aim of such a model is to gain knowledge about the represented system, explaining some of its phenomena, or help designing it. Mathematical modeling is a vast scientific and engineering field and is therefore not possible to explain in detail in one single book, let alone in one paper. It is used in countless scientific, engineering and even social studies and represents an important field in the study of the effects of the electromagnetic fields and accompanying coupled phenomena on cells, tissues and organs (Fear and Stuchly, 1998; Debruin and Krassowska, 1999; Debruin and Krassowska, 1999a).

As biological systems are often geometrically highly intricate, using analytical methods is, in most cases, not an option. Therefore, numerical methods are applied, the most widely used method being finite element modeling, a relatively simple but powerful tool for the analysis and the explanation of the processes taking place inside the biological systems.

Numerical models of cell and tissue electroporation proved useful for description of the underlying processes, as well as for evaluation of various influential parameters. Validated by experiments, they allow prediction of the outcome of pulse application and help in choosing the most efficient protocols and pulse parameters for specific applications. This approach also facilitates development and optimization of electrodes and their placement with respect to target tissue and can be of great help in treatment planning (Semrov and Miklavcic, 1998; Brandisky and Daskalov, 1999; Miklavcic *et al.*, 2000; Dev *et al.*, 2003; Miklavcic *et al.*, 2006a; Sel *et al.*, 2007; Corovic *et al.*, 2008; Zupanic *et al.*, 2008). A good model verified with experimental results is a powerful tool and offers useful insight into the understanding of processes modeled. However, we have to be aware of the fact that the processes involved are much more complex and the model is merely a simplified representation that can not replace experimental work. Nevertheless, it serves as a source of extra information and helps us to plan future experiments. Experimenting with such models is easier and sometimes the only possible or ethically acceptable alternative to experimenting on real biological systems.

In continuation, we explain basic aspects of finite element numerical modeling important in constructing numerical models of tissue electroporation, describe

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some characteristics of biological tissues and give some reference to the validation of results at the end.

Which Modeling Method to Use?

Starting point of the modeling process is deciding on the mathematical approach to adequately describe the modeled system, by first acquiring enough observable and measurable information about it. Typically, more than one modeling approach is possible and choosing the most suitable one depends on the modeler's or end user's objective needs and personal preferences, as well as physical and geometrical characteristics of the modeled system. In some cases, however, using more than one modeling approach can be beneficial in terms of model verification and validation. More than one phenomenon can determine a system, which is especially the case with biological systems. Generalizations and simplifications are possible and, in most cases, cannot be avoided. The model can refer only to some aspects of the real system, while disregarding the ones that either have a very limited influence on its accuracy or are out of scope of this particular model. Prior to building a model, we need to define its scope, apply necessary simplifications while being aware of the circumstances or the range of input variables the model is valid for.

Deciding on the right level of complexity for our model is not always an easy task as it involves a trade-off between simplicity and accuracy of the model. As a general guideline, if we are choosing from different models, giving comparable results, the simplest one is the most desirable. Namely, we need to be aware that adding complexity can make the model difficult to understand and experiment with and can pose computational problems.

Methods for solving partial differential equations: Analytical methods are rather complicated and are only feasible for use on problems where the geometry, material properties and boundary conditions can easily be described in a defined coordinate system (Cartesian, cylindrical or spherical). Simple analytical models can have certain advantages over numerical models. First, the input data needed is typically less extensive than that of numerical models. Also, analytical solutions have no numerical and discretization errors. The obvious limitation of analytical models is that only simple and uniform geometries, boundary and initial conditions can easily be modeled. In the last decades, however, analytical models have mostly been replaced by numerical models based on boundary element, finite difference, finite volume or finite element methods, due to the miniaturization and accessibility of both computer hardware and software. Of these methods, the latter is the preferred one to be used in modeling of biological systems, due to its easy implementation and its ability to handle more intricate geometries. The principle behind this method is the discretization of the geometry into smaller elements where the quantity to be determined is approximated with a function or is assumed to be constant throughout the element. Discrete elements can be of different shapes and sizes, which allows modeling of intricate geometries. In such models, the excitations can be changed easily, being that it only involves changing the boundary conditions on the same model. The model geometry, however, takes time and precision to be built and generalizations and simplifications need to be used when possible.

Finite Element Method

The finite elements method (FEM) turned out to be a very useful method for solving partial differential equations when studying electric field distributions inside biological systems. The essence of the method is the discretization of the geometry into smaller elements – finite elements – where the quantity of interest is approximated with a simple function or is assumed to be constant throughout the element (Reddy, 2004). Material properties inside each finite element are homogeneous. Mathematically, the finite element method is used for finding an approximate solution of partial differential equations (PDE) as well as of integral equations such as the heat transport equation. The solution approach is based either on eliminating the differential equation completely (steady state problems), or rendering the PDE into an equivalent ordinary differential equation, which is then solved using standard techniques such as finite differences, etc.

In solving partial differential equations, the primary challenge is to create an equation which approximates the phenomenon to be studied, but which is numerically stable, meaning that errors in the input data and intermediate computations do not accumulate and cause the resulting output to be meaningless.

In order to design a numerical model of a physical structure, the modeler must decide the appropriate resolution for modeling each component part, a task requiring considerable expertise and experience. After the right geometrical representation has been found, one of the next steps is the discretization of the geometry into finite elements – meshing. Too fine a mesh will cause unnecessary computational overheads when running the model, whereas too coarse a mesh will produce intolerable approximation errors. A simple rule is to choose a better resolution in the regions where we expect large gradients of the computed quantities or in the regions of our interest. Thin layers and small structures inevitably lead to a greater number of finite elements, as the mesh inside and around them needs to be denser. Further, borders between regions with very different material properties represent an additional problem, which again calls for higher mesh resolution. On the other hand, we are limited by computer capabilities and reducing the error by increasing the mesh resolution requires more computer time and the model can even end up being too complex to be solved. A simple way to optimize mesh density is changing its resolution and comparing the results given by models of different mesh densities. The optimal mesh is chosen as the least dense of those that yield similar results. Furthermore, using appropriate symmetries of the geometry can simplify our model a great deal.

Geometrical Considerations

When designing a numerical model, we must decide for the appropriate details to be included. Geometrically more detailed models will inevitably consume more of both, modeler's and computer time, but do not necessarily produce better quality of the results, as the inclusion of geometrical details depends on the purpose of the model. Further, taking advantage of geometrical symmetries of the system we are modeling can allow us to analyze a structure or a system by modeling only a portion of it while applying appropriate boundary conditions (see Figure 1a). This

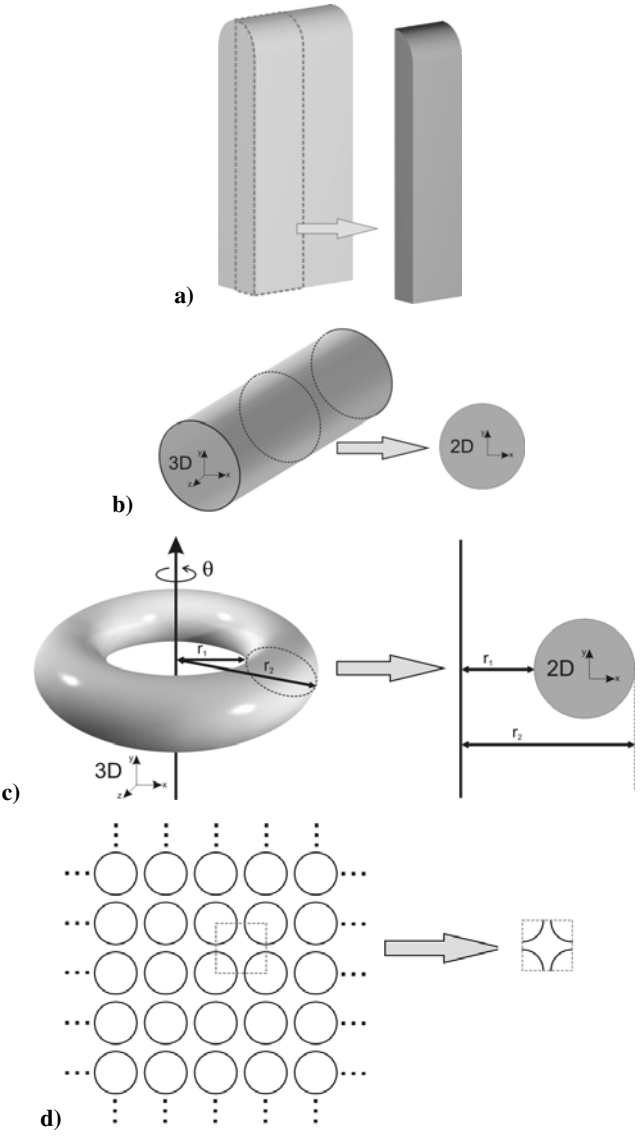
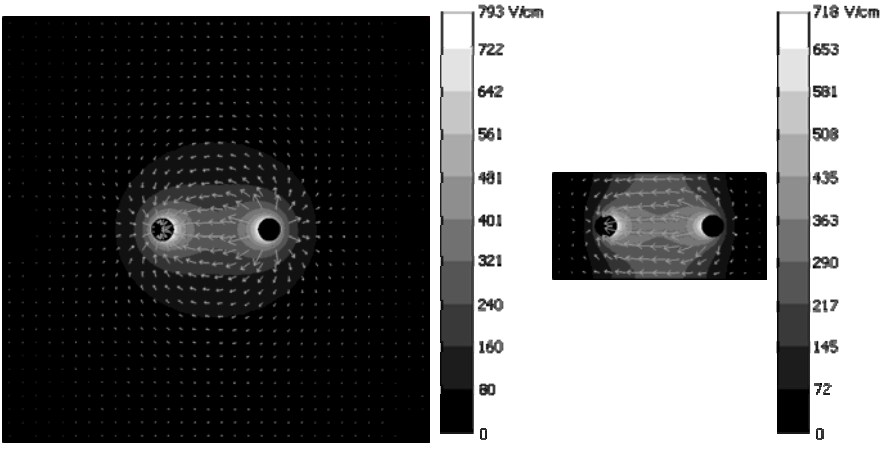


Fig. 1. Examples of using model symmetries to reduce the number of finite elements by reducing geometrical complexity. Taking advantage of such symmetries is only possible when the sources (in our case of electric current) exert the same symmetry. Boundary conditions on the section and symmetry planes and axes have to be set accordingly. a) Using only a quarter of the whole geometry to represent the whole structure. b) Two-dimensional representation of a three-dimensional geometry. In this case, the cylinder was represented by its cross-section. c) Two-dimensional representation of a three-dimensional axisymmetrical structure. d) A finite representation of an infinite two-dimensional lattice. The same approach can be used when modeling infinite structures in 3D.

approach can be used when both, the geometry as well as the sources (in our case of electric current) exert the same symmetry. It reduces the size of the model (the total number of nodes and elements), and consequently the analysis run time as well as the demands on computer resources. Alternatively, modeling only a portion of the whole geometry allows us to include more details in the model, when needed, thereby obtaining better results that would not have been possible with the full geometry. Similarly, sometimes we are able to represent a 3-dimensional structure by a single 2-dimensional plane. For example, in Cartesian coordinates, a structure stretched along a straight line (z-direction) can be represented with a structure in x-y plane, while the model is assumed to be uniform in the perpendicular z-direction (see Figure 1b). Similarly, if the structure is axisymmetrical, the plane of symmetry is the cross-section anywhere around the axis of symmetry. In this case, we are using a single 2-dimensional slice (r-z cylindrical coordinates) to represent the whole 360° of the structure (see Figure 1c). Further, in some cases, the geometrical structure we would like to model has a repetitive infinite or quasi-infinite pattern. Only a small portion of the whole array, a unit cell need be modeled by applying appropriate periodic boundary conditions (see Figure 1d).

In some cases the modeled system has no borders electrically insulating it from its surroundings; the electrical quantities are simply diminishing with increasing distance from the source. One such example is needle electrodes inserted in a tissue (Figure 2). In such cases, the outer boundaries of the model need to be far enough from the source(s), in order not to restrain the natural flow of the electric



(a) (b)

Fig. 2. The electric field distribution - E (white to black contours) and the electric current density - J (white arrows) in a homogeneous material, electric pulse is delivered through needle electrodes (the two black circles). The E and J distributions are shown in a section plane cut through the material, perpendicular to electrodes. a) The insulated borders of the model are far enough from the electrodes, which allows for the natural flow of the electric current, the electric field and the electric current near model borders are very close to zero. b) The borders of the model are too close to the electrodes, the electric field close to the border is not zero, electric current is artificially constrained.

current (See Figure 2a). Namely, when modeling such a system, the borders of the model are artificially electrically insulated from the surroundings. This effectively means that the boundary condition is set in such a way that no electric current flows in or out of the enclosing box – only tangential component of the electric current exists on the outer tissue borders while the normal component equals zero. If these borders are too close to the source(s) of the electric current, such as in Figure 2b, electric field and current distribution is deformed and does not reflect true situation.

Physical Considerations

After the geometry of the model has been constructed, the next step in the modeling process is setting the physics of the model, such as underlying equations, material properties, boundary and initial conditions. Commercially available finite element software packages usually have a great number of pre-defined application modes to choose from, already containing the equations relevant for the chosen physical problem. This makes the use of such software much easier for users with less mathematical knowledge. However, this may present a trap for beginners, as understanding the physics defining our problem is crucial for the whole modeling process, from geometry building, setting the underlying physics as well as interpretation and validation of the results. In continuation we shortly describe some important aspects of designing a numerical model to be used for studying the effects of electromagnetic fields on biological material.

Frequency dependent component

First, the material's response will be different when exposed to either direct (DC) or alternating current (AC). If our material is purely resistive, the system exerts no frequency dependency; the current is proportional to the voltage irrespective of the frequency. However, in general, materials have their capacitive or inductive component so the voltage to current ratio does depend on frequency and is termed impedance (Z). Impedance is a complex quantity, consisting of a resistance R (the real part) and a reactance X (the imaginary, frequency dependent part):

$$\vec{Z} = R + jX$$

The resistance can only be positive, while the reactance can be either positive (inductive character, current lagging behind voltage - X_L) or negative (capacitive character, voltage lagging behind current - X_C).

$$X_L = j\omega L$$

$$X_C = -j\frac{1}{\omega C}$$

Voltage and current must be regarded as vectors in the complex plane (phasors) that are out of phase, so the voltage to current ratio – the impedance – can also be given by its magnitude and phase angle:

$$\vec{Z} = |Z| \cdot e^{j\Theta}, \qquad \text{where}$$

$$|Z| = \sqrt{R^2 + X^2}$$

$$\Theta = arctg \, X/R$$

In summary, resistance is only a special case of impedance, when the material we are considering exerts no or negligible capacitive or inductive character ($jX=0$). Further, if our system is exposed to direct current, the frequency dependent part – the reactance X – plays no role when the system is in steady-state, after all the transients have faded out. It does, however, dictate the course of the transient of the system. Which poses the next question in the modeling process: Are we interested only in the steady-state of our system, or, are we studying transient phenomena – changes over time from $t=0$ until the system has reached its steady-state?

Transient vs. steady-state

Transient behavior occurs when the magnitude and direction of electrical quantities change with time. On the contrary, if they are constant with time throughout the entire volume, the system is already in its steady-state. To avoid any ambiguity, the steady-state does not mean absence of movement or flow in the system! If we take electric currents in a material as an example, it simply means that the "amount" of electric current in the system does not change within observed time; the magnitude of the current exiting the system equals the current magnitude flowing into the system at any time when in steady-state. In other words, time becomes an irrelevant variable for the analysis, since recently observed behavior of the system will continue into the future.

In many systems, steady state is not achieved until enough time has elapsed after the system is started or stimulated (externally or internally). The situation after the occurrence of the described changes of the system and before all internal quantities (states) of the system reach the steady-state is defined as a transient state. As an example, in an electrical system of purely resistive character, no transients occur at $t=0$, when the electrical stimulation is turned on. However, if the imaginary part (the reactance) is present in the impedance of the electrical system, its behavior exerts inertia, meaning that the change of electrical quantities in the system is not instantaneous. The capacitive or the inductive component opposes the sudden change at $t=0$ (applied voltage or current) and enforcing the transient state onto the system that will, however, eventually fade out.

Linearity

With respect to intrinsic characteristics of a system or equations describing it, an important consideration is whether the system is linear or nonlinear. Mathematically speaking, a linear function is one which satisfies both of the following properties: i) additivity $f(x+y)=f(x)+f(y)$; and ii) homogeneity: $f(ax)=af(x)$, where x and y are independent variables and a is any rational number. In other words, a nonlinear system does not satisfy the superposition principle stating that the response of a system caused by two or more input stimuli is the sum of the responses which would have

been caused by each stimulus individually. In terms of equations, a nonlinear system is any system where the variable(s) to be solved for cannot be written as a linear sum of independent components. Unfortunately, most physical systems are inherently nonlinear in their nature and, unfortunately, biological tissues are not an exception. Often, a physical property of a material is changed during a process (material's temperature coefficients, conductivity changes during tissue electroporation). Some typical types of nonlinearities often observed in nature are:

- Saturation: The condition in which, after a sufficient increase in an independent variable, its further increase produces no (or negligible) additional increase in the dependent variable (Figure 3a).
- Hysteresis: A phenomenon where the dependent and the independent variable bear a relationship which depends on prior history. More specifically, the response of the dependent variable (y) takes on different values for an increasing independent variable (x) than for a decreasing x (Figure 3b).
- Irreversibility: The irreversible processes cannot be restored to their original state once having undergone an irreversible change. Specifically, if we look at two possible states of the system – state A and state B, the transition between them is possible only in one direction (Figure 3c).

For some applications, a linear approximation of a nonlinear function can be found at (or around) a given point, for specific input values. However, if the model has to cover the whole (or larger) range of input values, the nonlinearities have to be considered in the model.

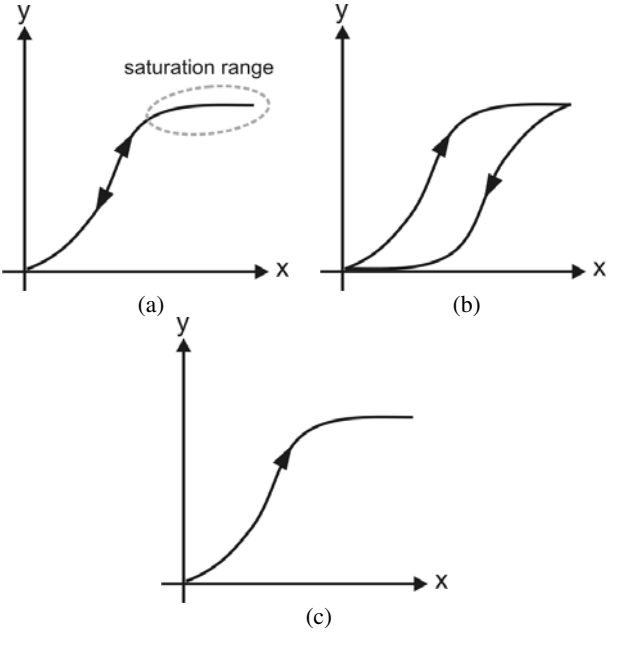


Fig. 3. Some typical nonlinearities: a) saturation b) hysteresis c) irreversibility

Multiphysics

The effects of various physical phenomena can be investigated by separately analyzing each individual phenomenon, without any regard to interaction between them. However, often we are dealing with two or more interacting, simultaneous phenomena, such as the coupling between the electric (E) and the magnetic field (B). An important coupling of physical phenomena in applications using electric pulses on biological tissues is heat transfer in tissue due to resistive heating (Tungjitkusolmun *et al.*, 2000). This coupling may give rise to tissue conductivity changes (due to temperature increase), which in turn changes the magnitude of electric current. When constructing a model, we have to estimate the influence of such interactions and, if needed to obtain accurate results, include mutual dependencies. To do so, we need data on how the material properties significant for one field (such as the electric field) vary with the value of another field (such as temperature) and vice versa.

Biological Tissues

Different biological materials perform different physiological functions so at their cellular and higher organizational level exert a number of interesting characteristics that have to be considered when representing them with a model. These differences are also clearly reflected in highly different bulk properties of biological materials (Gabriel S. *et al.*, 1996; Gabriel C. *et al.*1996; Miklavcic *et al.* 2006). They dictate the current densities and pathways that result from an applied stimulus and are thus very important in the analysis of a wide range of biomedical applications used for diagnosis and treatment. Biological tissues are inhomogeneous, nonlinear, and can, with respect to irreversible electroporation, exert saturation, hysteresis and irreversibility. We will further illuminate the theory presented so far for the case of biological tissues and give some examples.

Micro vs. bulk structure

Numerous examples could be given to illustrate either the importance or futility of including physiological details in the geometry of the model. Already at cell level, in vitro observations on cell suspensions can be represented numerically on different levels. For example, when studying the magnitude and the distribution of the electric field in a cell suspension, a material with homogeneous properties is an adequate model (Pavlin and Miklavcic, 2003). However, if the aim of our research is studying the phenomena on cell level or we are looking into interactions between cells, the influence of their size, shape, density and orientation, individual cells rather than bulk material have to be modeled (Susil *et al.*, 1998; Pavlin *et al.*, 2002; Valic *et al.*, 2003, Pucihar *et al.*, 2006, Pavlin and Miklavcic, 2008, Towhidi *et al.*, 2008). Even further, if we are using nanopulses, cell organelles must be added to the geometry (Kotnik and Miklavcic, 2006). Still, by using smart approaches, such as replacing a thin, non-zero conductivity cell membrane by a boundary condition between the cytoplasm and the exterior, getting rid of complexities while maintaining accuracy of the model is possible (Pucihar *et al.*, 2006).

Similar observations hold true on tissue level. Different tissues exert different levels of complexity and nonhomogeneity, however, including their particularities depends strongly on the purpose of the model. Skin, for example, is a very intricate tissue due to its highly inhomogeneous structure, leading to inhomogeneous electric properties. It consists of different layers, in terms of dimensions (thickness) and electrical properties: the outer thin layer of dead flat skin cells, the stratum corneum, the viable epidermis, dermis, and the subcutaneous tissue (Chizmadzhev *et al.*, 1998; Yamamoto and Yamamoto, 1976; Yamamoto and Yamamoto, 1976a). If the aim of the model is to study electroporation of skin as a target tissue, this layered structure needs to be included in our geometrical representation (Pavselj *et al.*, 2007). Moreover, even this bulk layered structure might sometimes prove inadequate. Smaller structures, such as hair follicles, sweat glands and blood vessels, or local transport regions as a result of skin electroporation (Pavselj *et al.*, 2008) may have to be added in order to study the processes on microscale, where they occur, and only then compare them to bulk observations. On the other hand, such details, unavoidably adding to overall complexity of the model, can be omitted in cases where skin electroporation is not studied directly, such as any application where electric pulses are delivered with external electrodes to tissues beneath skin (Pavselj *et al.*, 2005). However, other structures, such as major blood vessels may be important and need to be included in the model when studying electrochemotherapy of subcutaneous tumors. (Sersa *et al.*, 2008),

Alternatively, in cases where only one part of the whole system is either geometrically complex or needs better geometrical representation, the principle of embedded geometries can be used – the (usually more intricate) smaller part of the geometry is modeled separately and embedded in the model by properly setting the boundary conditions at the borders (Valic *et al.*, submitted).

Conductive and capacitive component of tissue

Electrical response of biological tissues when stimulated with DC electroporative pulses can be seen as quasi-stationary. Namely, for any material whose electric properties are in the range of those of biological tissues or organs and its dimensions do not exceed 1 m and the frequency of the electric field is below 1 kHz, the electrical behavior in any given moment as a response to electric current can be numerically described with a set of equations describing stationary fields. Although the impedance of biological tissue exerts capacitive component, electric field can be simplified as time independent thus the capacitive effects and the finite propagation of the electric current in the biological tissue are disregarded.

However, the transient of cell membrane charging may also be interesting to study. Namely, cell membrane charging time is in the order of microseconds, and typical pulses used for electropermeabilization of cell membrane are 100 microseconds long, with the amplitude of around 500 V/cm (Kotnik *et al.*, 1997; Kotnik *et al.*, 1998). It has been found that if pulses of much higher amplitude (e.g. 50 kV/cm) and much shorter duration are used – in the order of tens of nanoseconds – the charging effect also becomes pronounced on the membranes of intracellular organelles (Schoenbach *et al.*, 2001; Tekle *et al.*, 2005; Kotnik and Miklavcic, 2006). For a qualitative analysis of these processes, the time courses of organelle

and cell plasma membrane charging become important. Thus the capacitive component describing the electrical properties of the cell, its organelle(s) and their membranes can no longer be neglected.

Tissue anisotropy

When the properties of a material are the same in all directions, the material is said to be isotropic. However, some biological materials (most typical being skeletal muscle), are distinctly anisotropic. Therefore, when referring to published electrical property data, we need to know the orientation of the electrodes relative to the major axis of the tissue during impedance measurements (longitudinal, transversal, or a combination of both).

Tissue anisotropy is often related to the physiological demands made on the tissue. For example, skeletal muscles are composed of fibers that are very large, highly elongated individual cells and are aligned in the direction of muscle contraction. Electrical conduction along the length of the fiber is thus significantly easier than conduction between the fibers (the difference is about 7-fold) (Reilly, 1998). The longitudinal conductivity is significantly higher than the transverse conductivity, especially in the low frequency range. Tissue anisotropy is frequency-dependent (Hart *et al.*, 1999). Namely, if the frequency of the current is high enough, the anisotropic properties disappear (specifically for skeletal muscle, that happens in the MHz frequency range). At higher frequencies, charge movement takes place over shorter distances so large-scale structures become less important and capacitive coupling across membranes becomes more important.

A practical problem occurs when measuring the electrical properties of anisotropic materials: how to accurately align the applied electric field and tissue fibers. Namely, it has been shown that perfect alignment is crucial for obtaining accurate longitudinal and transverse values. A study on skeletal muscle tissue showed that a 5 degree misalignment from true perpendicular or parallel orientations would result in an 18% overestimate in the perpendicular direction and a 0.4% underestimate in the parallel direction when measuring conductivity (Epstein and Foster, 1983).

Nonlinearities of biological tissues

As stated previously, nonlinear natural processes are (unfortunately, from a modeler's standpoint) quite frequent in nature. If we speak strictly about tissue properties exposed to electric current, at least two important nonlinearities need to be considered.

One is the increase in tissue conductivity (σ) due to increased electric field (E) causing cell membrane electroporation (Pliquett and Weaver, 1996; Pavselj *et al.*, 2005; Sel *et al.*, 2005). This change of material properties has two more nonlinear characteristics. First, it is considered threshold phenomenon, meaning that the electric field has to reach a certain value, termed reversible electropermeabilization threshold E_{rev} in order to cause conductivity changes. Only then can we observe conductivity changes $\sigma(E)$ between the reversible and the irreversible value (too high an electric field, causing permanent cell damage) of the electric field. Its second characteristic is that, for the duration of the pulse, this conductivity change

is an irreversible phase transition process. More specifically, once the conductivity was increased in a given area of the tissue, it could not be changed back to its lower value during pulse delivery, even if the electric field strength drops below the threshold due to changed conductivities. Here, we would like to point out that one has to be careful to distinguish between the reversibility of cell electroporation (provided the electric field was below the irreversible threshold) *after* the cessation of pulsing; and the irreversible nature of the conductivity changes *during* pulse delivery – the change is only possible in one direction, tissue conductivity can only increase (Pavlin *et al*., 2005).

The second nonlinearity comes from the electrical-thermal coupling (Pliquett, 2003). Once a part of a tissue is permeabilized, it becomes more conductive and the current density increases several times, causing resistive heating. In turn, tissue conductivity increases even more, as most biological materials exert a positive temperature coefficient of electrical conductivity in the range of 1-3% $^{\circ}C^{-1}$ (Duck, 1990).

Threshold with respect to pulse parameters

Cell membrane is permeabilized when the threshold transmembrane potential is reached, thus when the external electric field is above the threshold value. This increased cell membrane permeability is reversible, provided the electric field is not too high. However, if cells are exposed to electric field above the irreversible threshold, they suffer permanent damage. For electroporation-based applications such as gene delivery (Golzio *et al.*, 2004; Andre *et al*., 2008) or transdermal drug delivery (Prausnitz, 1999; Denet and Preat, 2003; Denet *et al.*, 2004), this is not a desirable effect, as we need the cells to be viable after the treatment. On the other hand, for applications based on irreversible electroporation, we can irreversibly destroy target cells within a narrow range while leaving neighboring cells unaffected, representing a promising new treatment for cancer, heart disease and other disease states that require removal of tissue (tissue ablation) (Davalos *et al*., 2005; Lavee *et al*., 2007; Onik *et al*., 2007; Rubinsky *et al*., 2007).

In both cases, it is important to determine the needed amplitude of electric pulses at a given electrode-tissue set-up, to achieve an electric field distribution in the tissue, adequate for a given application. Electric field reversible and irreversible thresholds are both inherent characteristics of the tissue (also different for different tissues), no matter what kind of electrodes we use or if nonhomogeneous or composed tissues are involved. Of course, the electropermeabilization process as well as cell viability depend on electrical parameters, i.e. pulse amplitude, pulse duration and the number of pulses (see Figure 4). (Macek-Lebar *et al.*, 2002; Puc *et al.*, 2003).

However, accomplishing an adequate electric field distribution in the tissue is much more complex than merely calculating the voltage we need at given electrode separation (*U/d*). Mathematically, this ratio equals electric field only when delivering pulses to a homogeneous tissue through parallel plate electrodes whose surface is large (infinite) compared to separation between the electrodes

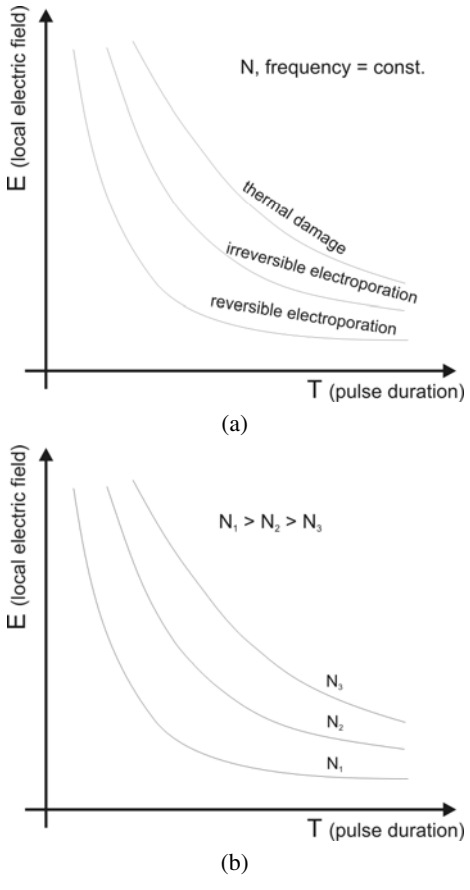


Fig. 4. Electroporation process is (for a given tissue-electrode geometry) controlled by pulse parameters. a) At constant number of pulses (N) and their frequency, lengthening pulse duration requires lower local electric field (pulse amplitude) for the same effect. If both are increased, the effects on the tissue become irreversible, or, at even higher values, tissue thermal damage can be observed, due to excessive resistive heating. b) Similarly, for any of the curves, if number of applied pulses is larger, the same effect can be achieved with a lower pulse amplitude and/or duration.

(see Figure 5a). It can still be used as an approximation of the electric field in the area away from the borders of the parallel plate electrodes of finite surface (Figure 5b). The *U/d* ratio is also often used to estimate the electric field between two parallel rows of needle electrodes; however, the approximation is very rough (Figure 5c). In the case of any other electrode geometry using plate, needle, microneedle or surface electrodes or if more than one tissue is involved, a numerical analysis has to be performed beforehand, as a part of treatment planning, in order to choose the right electrode configuration and the pulse amplitude.

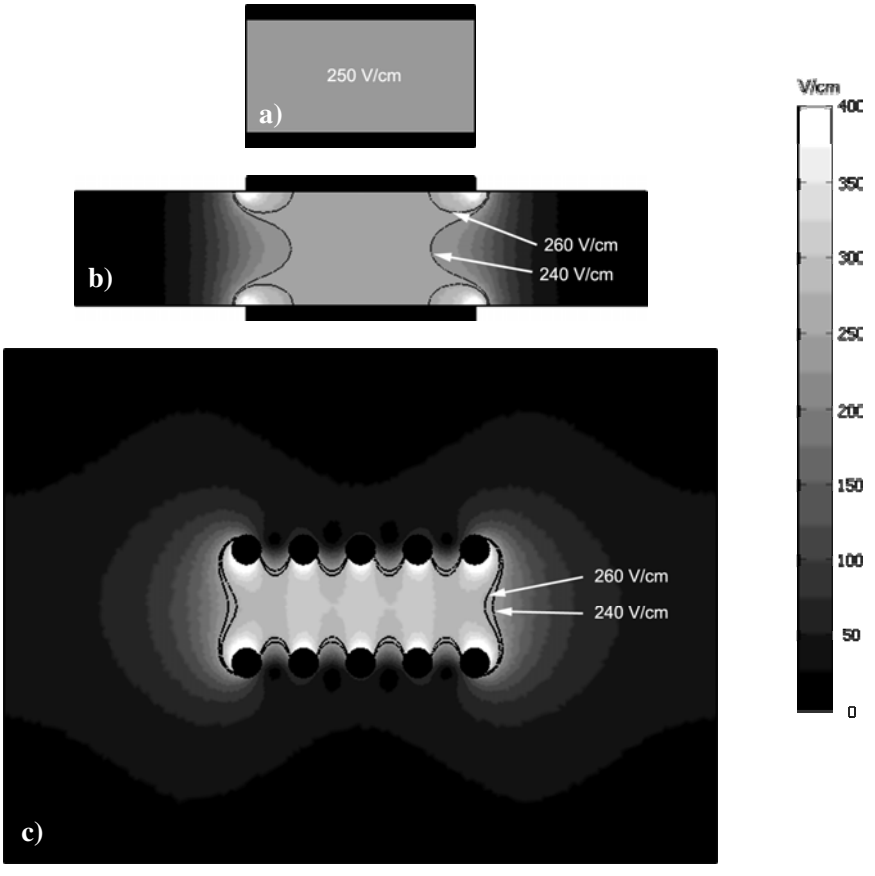


Fig. 5. Electric field distribution in a homogeneous tissue, values are given in V/cm in a section plane perpendicular to electrodes. a) The electric field equals the ratio U/d (voltage/distance between electrodes) only in the theoretical case where electric pulses are delivered through plate electrodes of infinite surface. Here, only a portion of this infinite structure is modeled with boundary conditions set to represent an infinite volume. The distance between the electrodes (d) is 4 mm, the applied voltage (U) is 100 V, and thus the electric field equals U/d=250 V/cm throughout the tissue between the electrodes. b) A real situation where electrodes are of finite surface. The electric field in the grey area between the two black iso-contours is between 240-260 V/cm, so the voltage to distance ratio in this area is a good approximation. The U/d approximation is valid in a greater portion of the tissue between the electrodes if the electrode surface is increased or the distance between them is smaller. c) Two rows of needle electrodes are used instead of plate electrodes. The length of the electrode array and the distance between the rows is the same as in b). The grey area between the two black iso-contours denoting electric field between 240-260 V/cm is very small and limited to a few narrow stripes. Throughout most of the area inside the electrode array is higher (around 300 V/cm or higher) and thus cannot be satisfactorily approximated by voltage to distance ratio.

Nonhomogeneous and composed tissues

When modeling electroporation of biological tissues, much consideration has to be given to the interpretation of the results in relation to possible simplifications in the model or inherent characteristics of different biological tissues. Namely, some simplifications might not have much effect in isotropic, homogeneous tissues, such as liver, but may yield useless results in nonhomogeneous, composed biological structures, such as layered skin or subcutaneous tumor, where electric field distribution is much more complex (Pavselj *et al.*, 2005; Pavselj and Miklavcic, 2007; Ivorra et al., 2008). To illustrate, when modeling electroporation in a homogeneous tissue, such as liver, the results are still useful and comparable to experimental data even if conductivity increase due to tissue electropermeabilization is neglected. In fact, early models did not take this nonlinear tissue behavior into account (Miklavcic *et al.*, 2000). However, when more complicated electrode-tissue set-ups were being studied with numerical models, experimentally observed phenomena could not be satisfactorily modeled in this way. Namely, upon applying electric pulses on a composed or layered tissue with a nonhomogeneous distribution of electrical conductivities, the voltage is divided among them proportionally to their electrical resistances (Pavselj and Miklavcic, 2008a). This leads to a more complex electric field distribution, meaning that some parts of the tissue, due to their low electrical conductivity (disproportionally lower than the rest of the tissue), are exposed to much stronger electric field. The electric field is the highest in the layer with the highest resistivity (lowest conductivity). In the case of the subcutaneous tumor this is the skin, which has the lowest electrical conductivity, and in the case of the skin fold, the highest electric field is in the non-conductive outermost skin layer, the stratum corneum. But more importantly, the electric field in the target tissues (tumor and viable skin layers) stays too low for successful electroporation. This fact raised the question of how is the experimentally confirmed successful permeabilization of the target tissues theoretically possible when external plate electrodes are used, which led to the inclusion of tissue conductivity changes in the numerical models.

Model Verification and Validation

The last, but nevertheless very important part of the modeling process is the verification and the validation of the constructed model, involving different aspects of evaluation. Mostly, these aspects should be taken into consideration from the very beginning of the process and can roughly be divided into three categories:

- Reaching the aim of the model (verification):
  Already in the planning phase of the modeling process, we have to set the scope of our model, the range of input data it should be valid for, as well as geometrical details to be included. However, as we build the model, some simplifications and trade-offs may have to be made. Comparing the actual result with the requirements set during the planning phase will tell us if our resulting model is still within the planned scope of the model.

- Explaining underlying physics (descriptive realism):
  In cases where almost nothing is known about the phenomena describing the modeled system, we are dealing with the so-called black box problem that can only be treated in terms of its input and output characteristics. Our only option may be finding a curve which has the best fit to a series of data points, while respecting possible constraints, without actual physical reference to the described process(es). However, different techniques of system identification (Ljung, 1999) can be applied in order to identify the physics defining our "black box", which can then be modeled. Namely, the purpose of models is gaining insight and explaining underlying phenomena, as well as using them for predicting the output at certain input data set. We should therefore direct our efforts to turn the black box into a set of equations if possible. Once again, as some trade-offs will most likely be necessary, we should assess if the modeled physical phenomena successfully explain the most important experimental observations.
- Agreement with empirical data (validation):
  One way to justify the physics used in the model (sometimes the only way) is comparing the output data obtained from the model to experimental data. Usually, or ideally, the experimental data can be divided into two groups: the training data and the validation data. The former is used to identify the process and to construct a model with its relevant parameters and constraints, while the latter is used to assess if the model is valid for any range of input parameters within the defined constraints.

Summary

Numerical modeling of cell and tissue electroporation and its accompanying phenomena has proven to be an efficient tool for the analysis and explanation of experimental observations. Moreover, it is indispensable in optimizing electrode geometries and electroporation protocols. Namely, every electroporation-based application has its own requirements and constraints. For gene electrotransfer, the cells in the treated tissue need to be viable after treatment in order to achieve high level of gene expression. This is not as important in electrochemotherapy, where killing cancerous cells is our aim. For this application, covering the entire volume of the treated tumor with a sufficiently high electric field, even if it exceeds the irreversible electroporation threshold, is of utmost importance. However, if the treated part is in the vicinity of any delicate tissue, organ or a part of an organ whose permanent damage has to be avoided at all cost, the protocol has to be carefully planned beforehand. The same goes for tissue ablation protocols using irreversible electroporation. Furthermore, any unwanted side effects, such as muscle contraction, pain or excessive tissue heating, should be minimized (Pliquett, 2003; Zupanic *et al.*, 2007; Davalos and Rubinsky, 2008). And last but not least, our efforts should also be directed towards treatment cost minimization in both, clinical as well as experimental environment.

For computer-aided optimization and treatment planning, the right representation of the real system has to be found, unavoidably including simplifications and trade-offs between simplicity and accuracy of the model (For more on irreversible electroporation treatment planning, see the chapter by Zupanic and Miklavcic.). It is impossible to construct a single model covering all aspects of cell and tissue electroporation, from subcellular level (cell organelle permeabilization using nanosecond pulses), through cell level (cell plasma membrane charging), to tissue level that again may or may not include details and tissue substructures. Further, when alternating current is used or when studying transients of the modeled system, the capacitive component of tissue electrical properties has to be taken into account. Nonlinearities, such as tissue conductivity increase during electropermeabilization must often be included in the model, as well as tissue resistive heating when longer pulses are used.

Although requirements and constraints of each application can be very different, careful analysis, parameter optimization and treatment planning is a common denominator to any electroporation-based application, allowing for efficient, safe and cost-effective procedure. With the increased accessibility and simplicity of computer software packages based on finite element method, carefully constructed numerical models can substantially contribute to increased efficiency and safety of experimental and clinical electroporation.

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