# NUMERICAL MODELS OF SKIN ELECTROPERMEABILIZATION

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**Abstract**

The application of electric pulses to biological cells causes the electroporation of cell membrane, increasing its permeability thus enabling the uptake of larger molecules that otherwise can not cross the membrane, such as drug molecules or DNA, into the cell. Electroporation can also be used to enhance transdermal drug delivery or DNA transfection in skin. The electropermeabilization process in skin was described theoretically, by means of numerical modeling, leaning on data derived from the in vivo experiments published previously. The numerical models took into account the layered structure of skin, macroscopical changes of its bulk electric properties during electroporation, as well as the creation of localized sites of increased molecular transport termed local transport regions (LTRs). The output of the models was compared with the current and the voltage measured during *in vivo* experiments and a good agreement was obtained. Also, comparing the voltages needed for a successful electropermeabilization of the skin fold as suggested by the model, with voltages achieving good *in vivo* gene transfection, good agreement can be observed. Finally, a comparison of our results with already published findings on skin electropermeabilization showed that the voltage amplitudes suggested by the model are also well in the range of the voltage amplitudes reported by other authors to cause skin permeabilization. With the models presented we used the available data to explain the mechanism of the tissue electropermeabilization propagation beyond the initial conditions dictated by the tissue initial specific conductivities.

**Keywords: electroporation, finite element method, plate electrodes, local transport regions.**

**Presenting Author's biography**

Nataša Pavšelj. Her main research interests lie in the field of electroporation, including finite element numerical modeling of electric field distribution in different biological tissue setups (subcutaneous tumors, skin fold) and cell systems (multicellular spheroids) and comparison of the theoretical results with the experimental work. In the last couple of years, her research work is specifically oriented on understanding and describing the process of skin electropermeabilization, mostly involving numerical modeling as well as in vivo experimenting.

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# 1 Introduction

Cell membrane is, in general, impermeable for larger molecules; however, the application of electric pulses to cells, either in suspension or tissue, causes the electroporation of cell membrane, increasing its permeability and making it possible for larger molecules that otherwise can not cross the membrane, such as drug molecules or DNA, to enter the cell [1]. Electroporation can also be used to create aqueous pathways across the skin's outermost layer, the stratum corneum to enhance transdermal drug delivery or to enhance DNA transfection after intradermal and topical DNA delivery [2]. In the paper presented, the electropermeabilization process in skin is described theoretically, by means of numerical modeling, leaning on data derived from the literature and the in vivo experiments resulting from our past research [3].

When referring to its electric properties, skin is a very intricate tissue due to its highly inhomogeneous structure. Skin epidermis contains different layers, but the one that defines its electric properties the most is the highly resistive outermost layer, the stratum corneum, composed of dead, flat skin cells. Although very thin (typically around 20 μm), it contributes a great deal to the electric properties of the skin. Deeper skin layers all have much lower resistivities [4,5,6].

One phenomenon we can observe from the in vivo experimenting is the increase in tissue conductivity due to cell membrane electroporation [7]. Upon applying electric pulses on skin, the electric field in the stratum corneum is very high, while the electric field in deeper layers of the skin stays too low for a successful electropermeabilization. However, due to the changes of bulk electric properties of the tissues during electroporation and consequently changed electric field distribution, the skin layers below stratum corneum end up being subjected to an electric field high enough for a successful permeabilization. Further, it has been shown that the electropermeabilization and consequently the increase in the conductivity of the stratum corneum are not homogeneous throughout the electroporated area [8,9,10,11]. Molecular and ionic transport across the skin subjected to high voltage pulses is highly localized in the so called local transport regions (LTRs). Our numerical models took into account the layered structure of skin and changes of its bulk electric properties during electroporation, as observed in the in vivo experiments. Also, the microscopical aspect of the skin electropermeabilization – the local transport regions (LTRs) – was modeled with numerical models.

# 2 Methods

Electric field and current calculations were made by means of commercially available computer program COMSOL Multiphysics, version 3.3 (COMSOL, Los Angeles, CA, USA) based on finite element method. COMSOL Multiphysics is a powerful interactive environment for modeling and solving all kinds of scientific and engineering problems based on partial differential equations (PDEs). Models can be built through a flexible graphical user interface, or by script programming in the COMSOL Script language or in the MATLAB language.

# 3 Numerical models

## 3.1 Macroscopical model of a skin fold

Experiments show that the conductivity of a tissue changes during electroporation. We simulated this process with a numerical model, modeling tissue and electrode setups and pulse parameters used in the in vivo experiments made previously on rat skin [3]. Four layers of skin were modeled: stratum corneum, epidermis, dermis and the underlying layer of fat and connective tissue. The electrodes were modeled as a boundary condition. By using symmetry and applying proper boundary conditions, only one fourth of the whole geometry can be modeled as to avoid numerical problems due to the complexity of the model and computer memory limitations (see Fig. 1 a,b)

When the electric field is applied to the skin fold, almost the entire voltage drop is on the outermost layer of the skin called the stratum corneum, which has the lowest conductivity. However, because of the changes of bulk electric properties of the electropermeabilized tissues, after the electro-permeabilization of the stratum corneum, the electric field “penetrates” to the layers underneath it [12]. This process was modeled as an irreversible phase transition problem, taking into account the increase in tissue conductivity due to cell membrane electroporation. Namely, when the electric field exceeds the pre-defined threshold, tissue conductivity increases. This change subsequently causes the change of the electric field distribution and of the corresponding current. Therefore, the final solution has to be reached iteratively. The electric conductivity values and their changes during electroporation were taken from the literature and experiments, as well as the electric field electropermeabilization thresholds. It is difficult to get their exact values, due to the lack of measurements in this field. However, we used the data found in literature, as well as our own experiments to set those parameters.

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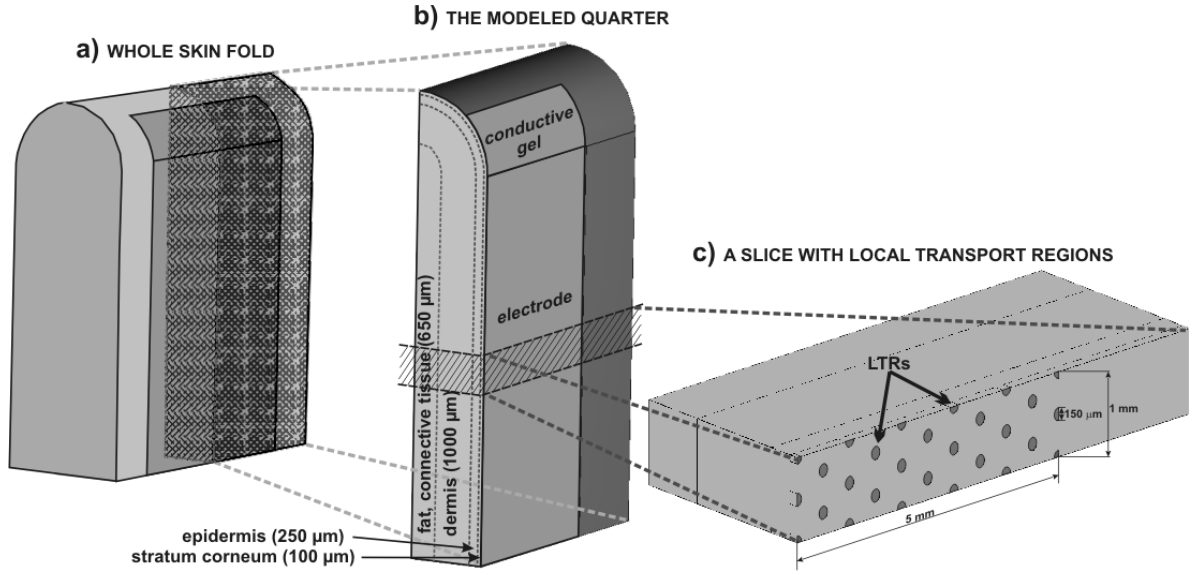


Fig. 1 The geometry of the whole skin fold modeled in COMSOL Multiphysics (a). Only one fourth of the skin fold was modeled to avoid numerical problems and save computer time (b). Further, numerical model of skin with local transport regions was described as a slice of the modeled quarter of a skin fold, using periodic boundary conditions (c).

Exactly how tissue conductivities (σ) change with electric field (E) is another unknown of tissue electropermeabilization. Due to the non-uniformity of the cell size and shape in the tissue, not all the cells are permeabilized at the same time once the threshold electric field is reached. Therefore, we assumed a gradual increase of the conductivities with electric field. In our model, the conductivities were increased from their low to their high values in four steps. The conductivity steps for all the skin layers followed an exponential dependence between 600 and 1400 V/cm of the electric field strength. The initial conductivities and the increased conductivities of the permeabilized tissues for all skin layers modeled are summarized in Tab. 1. Although the dermis and the epidermis were modeled as separate layers, the same conductivity was assigned to both, due to the lack of impedance data on different skin layers.

Tab. 1: The initial and the increased tissue conductivities used in the model.

| | initial conductivity | permeabilized conductivity |
|---|---|---|
| **subcutaneous layer** | 0.05 S/m | 0.2 S/m |
| **dermis, epidermis** | 0.2 S/m | 0.8 S/m |
| **stratum corneum** | 0.0005 S/m | 0.5 S/m |

### 3.2 Skin fold model with local transport regions

It has been shown that molecular and ionic transport across skin subjected to high voltage pulses is highly localized. The localized sites of molecular transport are called local transport regions (LTRs) [8,9,10,11]. We made another numerical model of skin electropermeabilization, where local transport regions were modeled as highly conductive structures in the stratum corneum. The results obtained from this model were compared to the results from the macroscopical skin fold model and the in vivo data previously published [3].

In the model where local transport regions were introduced, the same geometry as described in the previous subchapter was used, consisting of four layers of skin: stratum corneum, epidermis, dermis and subcutaneous layer of fat with connective tissue. The thicknesses of the skin layers were the same as in the skin fold model without LTRs, however, only a slice of the skin fold was modeled and periodic boundary conditions were set as to model the whole skin fold while simplifying the otherwise intricate geometry including local transport regions (Fig. 1c). The same nonlinear σ(E) dependences as well as the conductivity values before and after permeabilization as in skin fold model were used for all the skin layers except stratum corneum. As we made no attempt on visualizing local transport regions during our in vivo experiments, we took the data on LTR size and density and their conductivity from the literature [8,9,10,11]. The LTR and stratum corneum parameters are summarized in Tab. 2.

 

Tab. 2: LTR and stratum corneum (SC) parameters

| | |
|---|---|
| **LTR size** | 150 μm in diameter |
| **LTR density** | 40 LTRs per 0.1 $cm^2$ |
| **LTR covered surface** | 7 % of the SC surface beneath the electrode |
| **non-permeabilized SC conductivity** | 0.0005 S/m |
| **LTR conductivity** | 1.25 S/m (2500 times higher than non-permeabilized SC) |
| **average SC conductivity** | 0.09 S/m |

## 4 Results and discussion

In our in vivo experiments [3] we tried different pulse amplitudes. Five different voltages were used to permeabilize the skin – 160 V, 280 V, 400 V, 520 V and 700 V. During the pulse, the voltage between the electrodes and the current through the skin fold were measured. Our numerical models were also solved for the above voltages and the electric currents given by the models were compared with our experimental data. Fig. 2 shows the currents of the two models compared to the currents measured in vivo during the pulse.

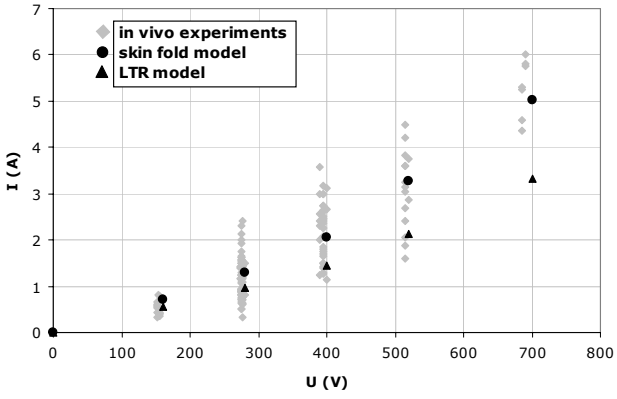


Fig. 2 Currents measured during the pulse, compared to the currents given by the models, with respect to the applied voltages.

As we can see, the current-voltage dependence of the macroscopical skin fold model seems to agree very well with the current-voltage dependence we got from the experiments. However, the electric currents given by the LTR model are shifted towards somewhat lower values (1.3-1.5 times). The reason for that can be attributed partially to different average conductivities of the permeabilized stratum corneum of both models. Namely, in the macroscopical skin fold model, the permeabilized part of the stratum corneum (mainly underneath the applied voltage boundary condition) has a higher value as opposed to the average conductivity of the permeabilized part of the stratum corneum in the LTR model (0.5 S/m vs. 0.09 S/m).

Also, the current-voltage dependence of the LTR model is more linear than that of the macroscopical model. In relation to this, it might be reasonable to construct different models for each applied voltage, with different number of local transport regions. Namely, as reported in the literature, the number of the LTRs increases with increasing voltage. In this way, the nonlinearity of the model with local transport regions embedded in the stratum corneum would be increased. Further, as the size, density and the conductivity of local transport regions were taken from the literature, where different electroporation protocols were used than in our in vivo experiments, the parameters of the local transport regions formed because of electroporation in our experiments might have been different. Namely, trains of exponentially decaying pulses were used by the researchers reporting the visualization of the local transport regions, while only one square electroporative pulse was used in our experiments. Also, the tissue-electrode geometry was different and, more importantly, heat-separated human cadaver epidermis was used in their experiments, while our experiments were done in vivo on rat skin. None the less, the electric currents of the LTR model are well in the range of those of the whole skin fold model and the in vivo experiments.

Further, we looked at the voltages needed for a successful electropermeabilization of the skin fold as suggested by the models, with voltages achieving good in vivo gene transfection (results published in [3], data not shown). For the comparison, we chose the voltage of 400 V between the plate electrodes, as this voltage resulted in a successful gene transfer in vivo. Electric field distributions represented with slice plots in 3D at the beginning and at the end of the electropermeabilization process for the applied voltage of 400 V are presented in Fig. 3. The figures show the area between the electric field strength of 600 – 1400 V/cm, that is, in the range where the conductivity increase was predicted in the model (between blue and red colors). In the blue areas the electric field was below the 600 V/cm, while the red color shows the areas above 1400 V/cm of the electric field strength.

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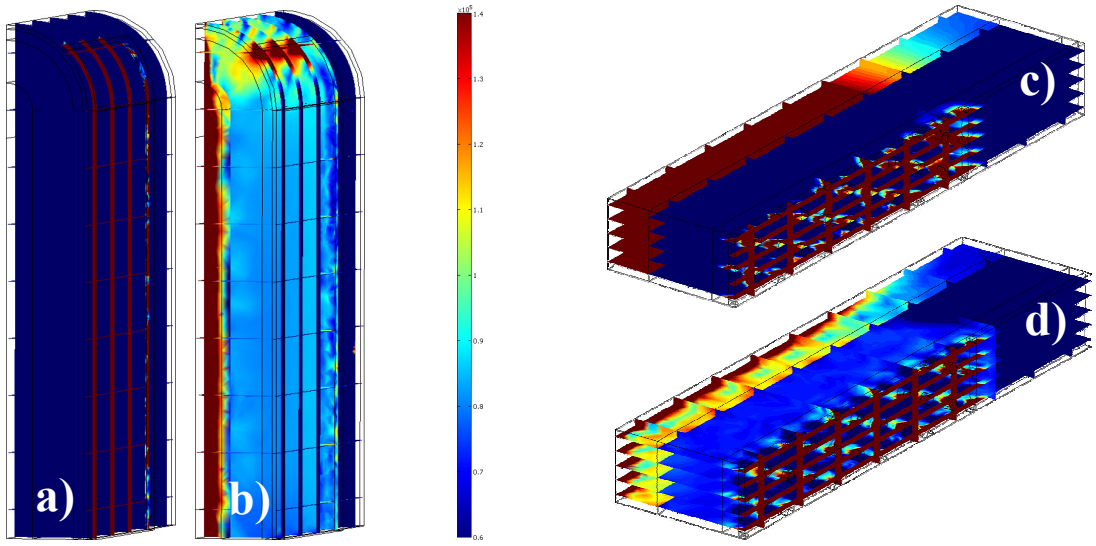


Fig. 3 3D slice plots of the electric field distributions during the electropermeabilization process in the macroscopical skin fold model (a) at the beginning of the process, b) at the end of the process) and in the LTR model (c) at the beginning of the process, d) at the end of the process). The electric field is shown in V/m. Blue to red colors: the electric field between 600-1400 V/cm; blue areas: E < 600 V/cm; red areas E > 1400 V/cm.

Fig. 3a and 3c show the electric field distribution at the beginning, when we apply voltage to the skin fold, for macroscopical and LTR models, respectively. All skin layers are non-permeabilized at this stage, meaning they still have their initial conductivities. The first tissue to get permeabilized is the stratum corneum. The increased conductivity of the stratum corneum and consequently changed electric field distribution enables the permeabilization of the underlying layers, the viable epidermis and the dermis, target layers for gene transfection. As suggested by the plots in Figure 3b and 3d, the target layers get permeabilized due to the conductivity changes of the permeabilized tissues, with the level of permeabilization being the highest in the topmost part of the skin fold (Fig. 3b). The results of the LTR model also show that the inclusion of the highly conductive areas in the stratum corneum leads to a nearly homogeneous permeabilization of the viable epidermis and the dermis, even though the conductivity increase in the stratum corneum is not homogeneous (Fig. 3d). Comparing the LTR model with the macroscopical model, 400 V seems to result in somewhat lower level of tissue permeabilization in the former. The electric field distribution is shifted to somewhat lower levels when local transport regions are modeled in the stratum corneum, however, the differences are small.

Comparing the voltages needed for a successful electropermeabilization of the skin fold as suggested by the model, with voltages achieving efficient in vivo gene transfection (data not shown, some results published in [3]) a good agreement is observed. In vivo experiments showed a lower expression when 160 V was used because the electric field in the viable epidermis and the dermis is still too low, as confirmed by the model (data not shown). Similarly, the in vivo expression was lower when 520 V or 700 V were used, due to lower cell viability caused by damaging high electric fields, which is again consistent with the results of the model, showing a very high electric field throughout the model at those voltages (data not shown).

Further, we compared the electric fields given by the models at the end of the permeabilization process (Fig. 3) and the calculated approximate electric field between the electrodes during in vivo experiments (voltage to electrode distance ratio), with already published findings on skin electropermeabilization. The voltage amplitudes resulting in a successful skin permeabilization during our in vivo experiments and the electric fields in the tissue given by the models agree well with the data we found in the literature [13].

## 5 Conclusion

Skin is an attractive target tissue for applications such as transdermal drug delivery and in vivo gene delivery. However, its low permeability presents a formidable obstacle and limits the number of drugs that can be delivered transdermally. Various in vivo experiments show a successful transdermal drug delivery or DNA delivery to the dermis and the viable epidermis by means of electroporation. Theoretically, the ratios of the specific conductivities of the skin layers suggest that the highest voltage drop when applying electric pulses on skin rests across the stratum corneum, while the electric field in the layers

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below is too low for a successful permeabilization. However, the electric conductivities of tissues subjected to electric pulses increase. As a result, the electric field "penetrates" deeper into the skin and permeabilizes the target cells. Further, molecular and ionic transport across the skin subjected to high voltage pulses was found to be highly localized in areas termed local transport regions (LTRs). We made numerical models describing these phenomena, based on the finite element method.

The output of the models was compared with the current and the voltage measured during in vivo experiments and a good agreement was obtained. Also, comparing the voltages needed for a successful electropermeabilization of the skin fold as suggested by the model, with voltages achieving good in vivo gene transfection in our in vivo experiments, good agreement can be observed. Finally, our findings also coincide well with results on skin electro-permeabilization published by other authors.

Some simplifications of the geometries of the numerical models were necessary due to their complexity. First, the stratum corneum was modeled six times thicker than in real skin and second, not enough skin fold volume was modeled at the base of the skin fold, thus the natural electrical current flow was somewhat restrained. Third, with the periodic boundary conditions set on the boundaries of the skin fold slice with LTRs, an infinite array of slices was modeled. An error was thus made, as the electric field distribution around the borders of the electrodes differs from the distribution in the tissue situated well in the middle of the electrode. The reason for these simplifications was the large number of the finite elements and the degrees of freedom of the models as well as large differences in skin layer thicknesses which made the computation difficult and long. However, the errors thus made were small enough.

With the models presented we used the available data to explain the mechanism of the tissue electropermeabilization propagation beyond the initial conditions dictated by the tissue initial specific conductivities.

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