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REVIEW

Mechanistic view of skin electroporation – models and dosimetry for successful applications: an expert review

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ABSTRACT

Introduction: Skin electroporation is a promising treatment for transdermal drug delivery, gene electrotransfer, skin rejuvenation, electrochemotherapy, and wound disinfection. Although a considerable amount of *in vitro* and *in vivo* studies exists, the translation to clinics is not as fast as one would hope. We hypothesize the reason lies in the inadequate dosimetry, i.e. electrode configurations, pulse parameters, and pulse generators used. We suggest adequate dosimetry can be determined by mathematical modeling which would allow comparison of protocols and facilitate translation into clinics.

Areas covered: We introduce the mechanisms and applications of skin electroporation, present existing mathematical models and compare the influence of different model parameters. We review electrodes and pulse generators, prototypes, as well as commercially available models.

Expert opinion: The reasons for slow translation of skin electroporation treatments into clinics lie in uncontrolled and inadequate dosimetry, poor reporting rendering comparisons between studies difficult, and significant differences in animal and human skin morphology often dismissed in reports. Mathematical models enable comparison of studies, however, when the parameters of the pulses and electrode configuration are not adequately reported, as is often the case, comparisons are difficult, if not impossible. For each skin electroporation treatment, systematic studies determining optimal parameters should be performed and treatment parameters standardized.

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1. Introduction

When biological cells are exposed to short high-voltage pulses, electroporation occurs, i.e. pores are formed in the plasma membrane leading to transient permeability increase, and molecules, for which the cell membrane is usually impermeable, can pass across membrane [1]. Therefore, electroporation occurs due to high electric field imposed across a short distance (plasma membrane), i.e. above-threshold induced transmembrane voltage. In a similar way, the electric field across the *stratum corneum* (SC) causes electroporation of skin, i.e. application of electric pulses to the skin disrupts its barrier function by means of skin electroporation.

The main mechanism behind skin electroporation is thus the disruption of the most resistive and impenetrable layer of the skin, the SC. When short high-voltage pulses are applied, small aqueous pores, i.e. local transport regions (LTRs), are formed within the lipids of the SC (Figure 1) [2–5]. LTRs are areas of increased electric conductivity and permeability. When pulses are longer, in the millisecond range, Joule heating causes melting of the lipids around the edges of LTRs as well as around the preexisting defects and appendages (sweat glands, hair follicles) in the SC, the size of the defects increases and LTRs grow to a few hundred micrometers in diameter. These newly formed defects in the SC decrease its normally very high impedance and allow the electric field to penetrate lower layers of the skin [6,7], thus causing electroporation of the living cells beneath the SC. Additionally, electric pulses cause electrophoretic transport of charged molecules through the LTRs, assisting their transdermal delivery [8]. Electroporation should be distinguished from iontophoresis, which is the application of continuous direct low electric currents to the skin. The two main transport mechanisms of iontophoresis are electrophoresis (moving of charged molecules through skin) and electroosmosis (movement of neutral molecules by convective flow) [9]. In iontophoresis, no LTRs are formed in the SC, most of the transport occurs through preexisting defects and appendages. Iontophoresis is mostly used for transdermal delivery of small and charged molecules.

1.1. Applications of skin electroporation

Transdermal or intradermal gene electrotransfer [10] is one of the most widely used and promising skin electroporation applications where short-term gene expression is successfully achieved, for example in gene transfer of antigen-presenting cells in immunotherapy such as DNA vaccination [10] or in transfection of skin cells to produce various proteins [11]. Here, the aim is to transfect successfully cells in the skin. In transdermal drug delivery, however, the aim is to achieve transport of small therapeutic molecules across the skin, for example, to treat pain, dementia, Parkinson's disease [9,12–14], i.e. molecules are applied topically and have to penetrate through the skin and reach the microvasculature . In cosmetics, electroporation is used in the so-called 'needless

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Article highlights

- Mathematical models of skin electroporation facilitate our understanding of the phenomena, help to reveal relevant parameters for treatment efficacy, decrease the number of *in vitro* and *in vivo* experiments needed and enable predictions about the treatment efficacy.
- One of the reasons for slow translation of skin electroporation into clinics are non-standard pulse parameters, non-standard electrode configurations, generators not complying with their technical specifications or lacking technical specifications, poor or missing reporting on the delivered waveforms, electric field distribution, not performing the current–voltage measurements and significantly different skin structure of animals and humans.
- We suggest guidelines for reporting the dosimetry to be followed. The use of high-quality electroporation equipment, with reliable and traceable operation, together with controlled dosimetry and predictive modeling raises the quality of studies and enables faster development of the field and eventual translation into clinics.
- We propose that the pulse parameters and electrode configurations for skin electroporation should be determined and standardized with the use of mathematical models, taking into account the electric field distribution, electrical, thermal, and chemical damage, drug/plasmid distribution and other parameters, deemed relevant for the treatment.
- When designing new clinical as well as esthetic (for the use in cosmetics) pulse generators, these should comply with existing standards. In Europe, they have to comply with a Medical Device Regulation MDR 2017/745, and in the USA, the device should be approved by the FDA (Food and Drug Administration). Also, new standards specific to electroporation devices should be developed.

This box summarizes the key points contained in the article.

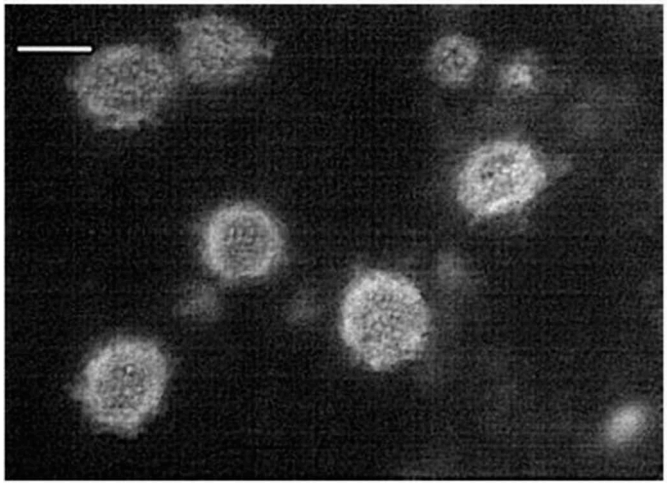


Figure 1. Mechanism of skin electroporation. After high-voltage electric pulses are applied, local transport regions form through which the transport of larger molecules can occur. Here, local transport regions are imaged via calcein transport. Reprinted from Bioelectrochemistry and Bioenergetics, 47/1, Pliquett et al., Local transport regions (LTRs) in human stratum corneum due to long and short `high voltage' pulses, 151–161, Copyright (1998), with permission from Elsevier.

mesotherapy' or 'mesoporation' to rejuvenate the skin [15–17], although our measurements of some devices indicate that instead of electroporation pulses, low-intensity radiofrequency (RF) pulses are delivered. In classical electroporation short, square wave, high-voltage electric pulses are delivered to the tissue and the treatment is considered being 'non-thermal'. Pulses of irreversible electroporation are applied to remove the aged cells and to promote the growth of new cells, while extracellular matrix remains undamaged [18], and in treatment of superficial wound disinfection to kill the antibiotic-resistant bacteria [19,20]. IRE is ablative treatment, while in electrochemotherapy, the aim is to achieve reversible electroporation to maximize the differential effect of chemotherapeutic drug on fast-dividing, i.e. tumor cells. Cutaneous and subcutaneous tumors are treated with a combination of electric pulses and chemotherapeutic drug [21,22], and also keloids and hypertrophic scars can be treated with intralesional bleomycin injection combined with electroporation when other treatments have failed [23].

Although skin electroporation lends itself as a promising approach for transdermal drug and gene delivery, the translation into the clinical setting is lagging behind the *in vitro* and *in vivo* studies [14]. In our review, we critically assess the existing applications of skin electroporation, discuss possible reasons for this relatively slow transfer of skin electroporation into clinical use and suggest possible solutions for improving it.

1.2. Models of skin electroporation

As the experimental setups of skin electroporation can vary significantly, direct comparison of results obtained by different electrode configurations and pulse generators are difficult if not impossible without modeling and/or more accurate dosimetry or detailed description. The models of skin electroporation could be instrumental in comparing results, facilitating the translation from the *in vitro* to the *in vivo* and finally to the clinics as well as decreasing the number of experiments needed. Mathematical models of skin electroporation vary between each other depending on the desired outcome of the model. For example, they are 1) analytical or numerical, 2) take into account different physics (electrical, thermal, transport), 3) are at different spatial (molecules, cells, tissue) and 4) time scale (movement of molecules, formation of membrane pores, formation of LTRs), 5) validated or not validated etc. More detailed models describe thermal [24,25] or electric [6,7,17,26–30] effects or both coupled together [31–38], primarily for the description of changes in the *stratum corneum* (SC) based on mechanisms of skin electroporation (formation of the defects in the lipid bilayers of the SC, LTR formation, and growth [24,32,39], mechanical deformation of the SC [30]). In the treatment planning of electroporation-based medical treatments, models have been used to calculate the electric field and thermal damage in the cutaneous/subcutaneous tumors and surrounding tissue [29,31,37,40–43], although for standard electrodes and pulses, the standard operating protocols obviate the need to calculate each case separately [21]. Electric and/or thermal models can further be coupled to transport models via the diffusion and electrophoresis through the LTRs [32,33,44–47], the dual-porosity model [48], compartmental models [45] and/or regression models [49]. In case of the DNA transport, the electric properties of the injected plasmid DNA were taken into account [50], the efficiency of gene electrotransfer was evaluated according to the predicted plasmid DNA concentration inside the reversibly electroporated tissue [51] as well as taking into account thermal stress

and tissue damage during gene electrotransfer [52], due to pulse delivery. The extravasation of macromolecules in the size of antibodies or plasmid DNA from blood vessels into the surrounding tissue during skin electroporation was also modeled [53]. Most of the models treat the skin as a bulk tissue of a few layers of different dielectric properties [7,27,29,33,46], or as an equivalent circuit [34,54], however, also multi-scale model, i.e. model which takes into account the microstructure of the skin is available [6]. Recently, a computational study at the level of single lipids in the SC was performed, i.e., a molecular dynamics study, which showed that aqueous pores indeed form in the SC [5]. Some typical examples of the models, progressing from the molecular level to elaborated mechanistic macroscale models, are shown in Figure 2. For example, in (a) the molecular dynamics study of pore formation, (b) the equivalent circuit, (c) electric field distribution in the bulk skin model, (d) model of a single LTR or (e) several LTRs in the SC, (f) single cells from the multi-scale model and (g) model of gene electrotransfer. Depending on the desired model output (electric field distribution, tissue heating, plasmid distribution, cell viability, pore formation, LTR formation, etc.), corresponding model should be used and/or coupled with others to add more functionalities or spatial/time scales to the calculation.

1.3. Pulse generators and dosimetry

In existing studies, various electrode configurations from invasive to noninvasive are used for pulse application [55]. In Table 1, we summarize custom-made, i.e. prototype electrodes used in different studies and similar commercially available electrodes that researchers could use as well. Pulses of different lengths, shapes, and voltages (schemes are shown in Figure 3) are applied with different pulse generators (electroporators), listed in Table 2. It needs to be emphasized that a clinical electroporator i. e. pulse generator is a medical device, which has to follow the requirements appointed by the local medical regulations and meet the medical device standard IEC 60601 (a series of technical standards for the safety and effectiveness of medical electrical equipment). In Europe, it has to comply with a Medical Device Regulation MDR 2017/745 (which in 2017 replaced Medical Device Directives (93/42/EEC, 98/79/EC and 90/385/EEC) and is in a transition period until May 2020) and in the USA, the device should be approved by the FDA (Food and Drug Administration) for specific indication. In Europe, the area of cosmetic devices has not been well regulated until now but with the new MDR (EU 2017/745), also esthetic devices, which present the same characteristics and risk profile as medical devices, are included under the scope of this Regulation.

The design, development, and quality assurance of an electroporator is challenging because the electrical characteristics of biological load vary between tissues, samples from one tissue and parts of the body and also vary with age and hydration on patients. SC has a significantly higher impedance than lower skin layers or muscle tissue, meaning that at the same electrode configuration and applied electric pulses the current between the electrodes is higher for invasive than for noninvasive electrodes. Additionally, the invasive and noninvasive electrodes present different risks and are therefore classified into different safety classes of the standards for medical devices. The electric field distribution is also electrode geometry dependent [84], while the effective parameter is a local electric field; thus, the comparison of different electrode types and transition from *in vitro* to *in vivo* to the clinical use is only possible through modeling.

2. Conclusion

Skin electroporation is a promising modality for treating different conditions with transdermal drug delivery, gene electrotransfer, electrochemotherapy, and irreversible electroporation. A considerable body of *in vitro* and *in vivo* literature exists, which use different electrode configurations, waveforms, and pulse generators. We presented some of the electrode configurations and pulse generators, prototype as well as commercially available, appearing in the literature and pointed out their advantages and drawbacks (see Table 2). Results from studies with different parameters are sometimes difficult (if not impossible) to be compared. We thus propose to use mathematical models, which can allow comparing different experimental results and enabling better treatment efficacy prediction. Alternatively, reporting should follow recommendation and provide detailed description of pulses and electrodes [85–87], that should allow development of models by experts.

3. Expert opinion

Skin electroporation is a promising method as it is safe, fast, and efficient method of delivering drugs or DNA across and into the skin and affecting skin structure. Unfortunately, the translation of skin electroporation protocols into clinics is not as fast as one would expect [14]. Already translation from the *in vitro* to the *in vivo* is difficult due to a significantly more complex environment *in vivo* and often different electrodes used. An interesting link between the *in vitro* and the *in vivo* studies is the *in vitro* 3D reconstructed human skin [88–90] which is more similar to *in vivo* human than rodent skin. Nevertheless, before entering into the clinics, the *in vivo* experiments must be up-scaled to humans, which can result in enormous amounts of drugs/plasmids needed, but often also requires scaling up of electrodes and pulse generators.

We identified two main reasons for the hampered transition of skin electroporation into the clinics. 1) *In vivo* studies are usually done on animals (rodents, pigs, rabbits) with significantly different skin structure than humans, which is recognized by the researchers, but they have no way of translating their results from animal to human skin. They differ in, among others, the thickness of the layers, their number, the density of the appendages, hydration. The structure of the skin varies significantly also between different people, among different body parts of one person or even of the same part of skin throughout the day. 2) The dosimetry of the delivered pulses is not well controlled. Pulses of various shapes, durations and amplitudes are applied to different electrode configurations with different pulse generators. Some electroporators have vague or non-existent technical specifications, meaning the researchers do not know exact pulse parameters. The

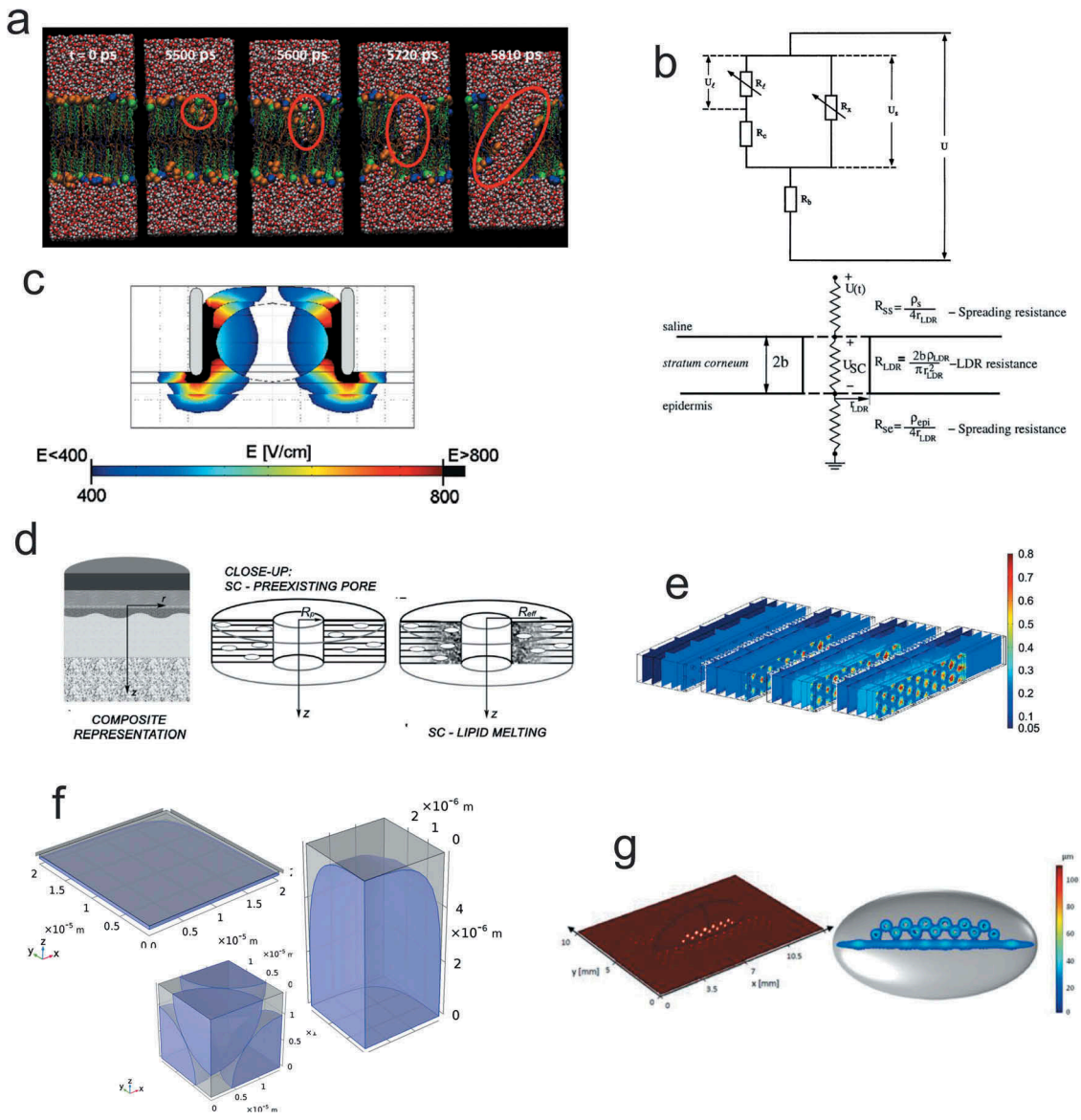


Figure 2. Examples of different models of skin electroporation. (a) Results of the molecular dynamics simulation of the pore formation in the stratum corneum. Reprinted with permission from Gupta R, Rai B. Electroporation of Skin Stratum Corneum Lipid Bilayer and Molecular Mechanism of Drug Transport: A Molecular Dynamics Study. Langmuir. 2018;34:5860–5870. Copyright (2018) American Chemical Society. (b) An equivalent circuit of the skin. Above: the entire SC is modeled with one equivalent circuit. R_b represents the resistance of the bulk solution. The resistance of the skin is represented by two parallel branches with R_x being the pathway through the appendages, R_l through lipid bilayers in the SC and R_c the inner resistance of each compartment of the model. (This model assumes that SC is made of many hydrophilic compartments, separated by boundary bilayers.) Reprinted from Biophysical Journal, 68/3, Chizmadzhev et al., Mechanism of electroinduced ionic species transport through a multilamellar lipid system, 749–765, Copyright (1995), with permission from Elsevier. Below: Only one LDR (local dissipation region) in the SC is modeled with the equivalent circuit. Reprinted from Bioelectrochemistry, 57/1, Martin et al., Theoretical analysis of localized heating in human skin subjected to high voltage pulses, 55–64, Copyright (2002), with permission from Elsevier. (c) A bulk numerical model of the subcutaneous tumor showing electric field distribution when 276 V are applied to parallel plate electrodes. Adapted from [91]. (d) The model of a single local transport region formation inside a layered skin model. Left: a layered model of the skin. Middle: The geometry of a preexisting pore inside the SC. Right: Melting of the lipids around the preexisting pore and consequent increase in the radius of the LTR. Reprinted from Journal of Biomechanical Engineering, 129/5, Becker SM and Kuznetsov AV, Local Temperature Rises Influence In Vivo Electroporation Pore Development: A Numerical Stratum Corneum Lipid Phase Transition Model, 712–721, Copyright (2007), with permission from Elsevier. (e) A bulk model of the skin with included different dielectric properties of each skin layer and with a model of LTR formation inside the SC. Four three-dimensional slice plots of the conductivity distributions (S/m) represent four stages of the process in chronological order when 400 V is applied. © [2008] IEEE. Reprinted, with permission, from Pavšelj N, Miklavčič D. Numerical Models of Skin Electropermeabilization Taking Into Account Conductivity Changes and the Presence of Local Transport Regions. IEEE Transactions on Plasma Science. 2008;36:1650–1658. (f) The geometry of the cells of the skin (corneocyte, keratinocyte, spheres in the papillary dermis) used in the multi-scale model of skin electroporation. From each cell, equivalent dielectric properties of the respective layer were obtained. © [2018] IEEE. Reprinted, with permission, from Dermol-Černe J, Miklavčič D. From Cell to Tissue Properties – Modeling Skin Electroporation With Pore and Local Transport Region Formation. IEEE Transactions on Biomedical Engineering. 2018;65:458–468. (g) A model of gene electrotransfer with subcutaneously injected plasmid after pulse application. Left: Skin patch with the injected plasmid. SC is shown in red with local conductive pathways in white. Right: Gray ellipsoid is the injected plasmid DNA. Electrophoretic movement of the plasmid DNA through reversibly electroporated volume of the skin tissue is shown as the trajectories of the plasmid DNA movement inside the volume of reversible electroporation due to the non-uniform electric field. © [2019] IEEE. Reprinted, with permission, from Forjanič et al. Electroporation-Induced Stress Response and Its Effect on Gene Electrotransfer Efficacy: In Vivo Imaging and Numerical Modeling. IEEE Trans. Biomed. Eng. 2019;66:2671–2683.

Table 1. For skin electroporation, different electrode configurations are used. Here, we show electrode types usually used for skin electroporation in *in vitro* as well as *in vivo* studies. Electrodes are divided into invasive and noninvasive, for each we also state the intended area of use, the manufacturer, the electrode configuration (number of electrodes and their dimensions), material, reference to the study or manufacturer's webpage and an image of the electrodes.

Electrode type	Area of use	Manufacturer	Electrode configuration (number of electrodes and their dimensions)	Material	Reference	Image
Noninvasive electrodes						
PLATE OR CALIPER ELECTRODES	Gene electrotransfer, transdermal drug delivery, electrochemotherapy, skin rejuvenation	BTX – Harvard Apparatus	2 1 × 1 cm, 1.5 × 1.5 cm, 2 × 2 cm	Brass or stainless steel (only 2 × 2 cm)	[56,57]	
		IGEA	2 8 mm gap/6 mm (older version) 10 mm x 30 mm x 0.8 m	medical grade steel	[50]	
		custom prototype	4 6 mm gap	NA	[58]	
		BTX – Harvard Apparatus	13 spaced 2 mm apart	gold plated	[59,60]	
L-SHAPED ELECTRODES	Transdermal drug delivery, gene electrotransfer, electrochemotherapy	Leroy BIOTECH	2 10 mm gap 10 mm x 3 mm	stainless steel	[61]	
		custom prototype	2 4 mm gap 20 mm x 1 mm	stainless steel	[62]	

(*Continued*)

Table 1. (Continued).

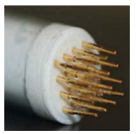
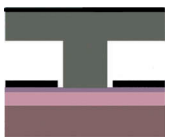
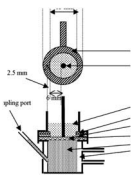
Electrode type	Area of use	Manufacturer	Electrode configuration (number of electrodes and their dimensions)	Material	Reference	Image
PIN SURFACE ELECTRODES	Gene electrotransfer,transdermal drug delivery	custom prototype	16 0.3 mm diameter Grid of 2 mm-apart pins	gold-plated	[63–65]	 Image from [65]
		Iskra Medical	7 spring-loaded pins in honeycomb configuration, spaced 3.5 mm apart	NA	[11,66]	
ELECTRODE- RESERVOIR DEVICE	Transdermal drug delivery	custom prototype	2 should not exceed 200 µm	NA	[67]	
INTERDIGITATED ELECTRODES	Gene electrotransfer	custom prototype	patch-like electroporation array	gold electrodes on pliable parylene substrate	[68]	 Image from [68]
RING AND NEEDLE ELECTRODES	Transdermal drug delivery	custom prototype	ring electrode: (outside diameter 25 mm and inside diameter 15 mm) needle electrode: 3 cm x 3 mm	silver	[102]	

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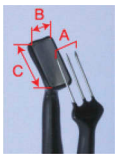
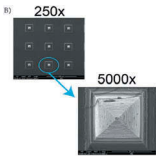
Table 1. (Continued).

Electrode type	Area of use	Manufacturer	Electrode configuration (number of electrodes and their dimensions)	Material	Reference	Image
MESO THERAPY ELECTRODES	Transdermal drug delivery	Derm Equipment	plate electrode 25 mm in diameter	NA	[17]	
Invasive electrodes NEEDLE ELECTRODES	Gene electrotransfer, electrochemotherapy	IGEA	7 hexagonal configuration Diameter 0.7 mm, length 10 mm, 20 mm or 30 mm	medical grade steel	[69]	
		IGEA	8 (2 rows of 4) linear configuration Diameter 0.7 mm, length 10 mm or 20 mm or 30 mm	medical grade steel	[70]	
		BTX – Harvard Apparatus	23 mm tip length ultra thin diameter	platinum	[71]	
		BTX – Harvard Apparatus	8 or 12 4 mm or 6 mm gap lengths: 2, 3, 5, 10, 16, 25,	stainless steel	[72]	
		Leroy	8 (two rows of four) Linear configuration 8 mm between two rows, each needle is 2 mm apart 0.88 mm diameter, 15 mm long	stainless steel	[61]	

(*Continued*)

Table 1. (Continued).

Electrode type	Area of use	Manufacturer	Electrode configuration (number of electrodes and their dimensions)	Material	Reference	Image
FORK ELECTRODES	Gene electrotransfer	BEX and NEAPGENE	4 (3 needles + 1 plate) 3 x 2.5 mm needles intervals, length 3 mm or 5 mm or 10 mm, diameter 0.5 mm	stainless steel, coated in platinum	[69,73,74]	
MICRONEEDLE ARRAY ELECTRODES	Gene electrotransfer	custom prototype	Pyramidal shape, radius of the tip is below 1 µm Needles length: 200 µm ± 7 µm (900 needles per array and 121 arrays per wafer. Needle spacing: 90 µm	solid silicon, glass, titanium, ceramic, polymer	[40,75]	 Image from [75]
GRID ELECTRODES	Electrochemotherapy	custom prototype	Flexible support and 67 needles, length 5 mm or 10 mm	stainless steel	[76]	 Image from [76].

NA: not available.

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L-shaped prototype electrodes: Reprinted from Journal of Controlled Release, 134/2, Mazères et al., Non-invasive contact electrodes for in vivo localized cutaneous electropulsation and associated drug and nucleic acid delivery, 125–131, Copyright (2009), with permission from Elsevier.

Ring and needle electrodes: Reprinted from Effect of electric field on the enhanced skin permeation of drugs by electroporation, 90/2, Mori et al., Effect of electric field on the enhanced skin permeation of drugs by electroporation, 171–179, Copyright (2003), with permission from Elsevier.

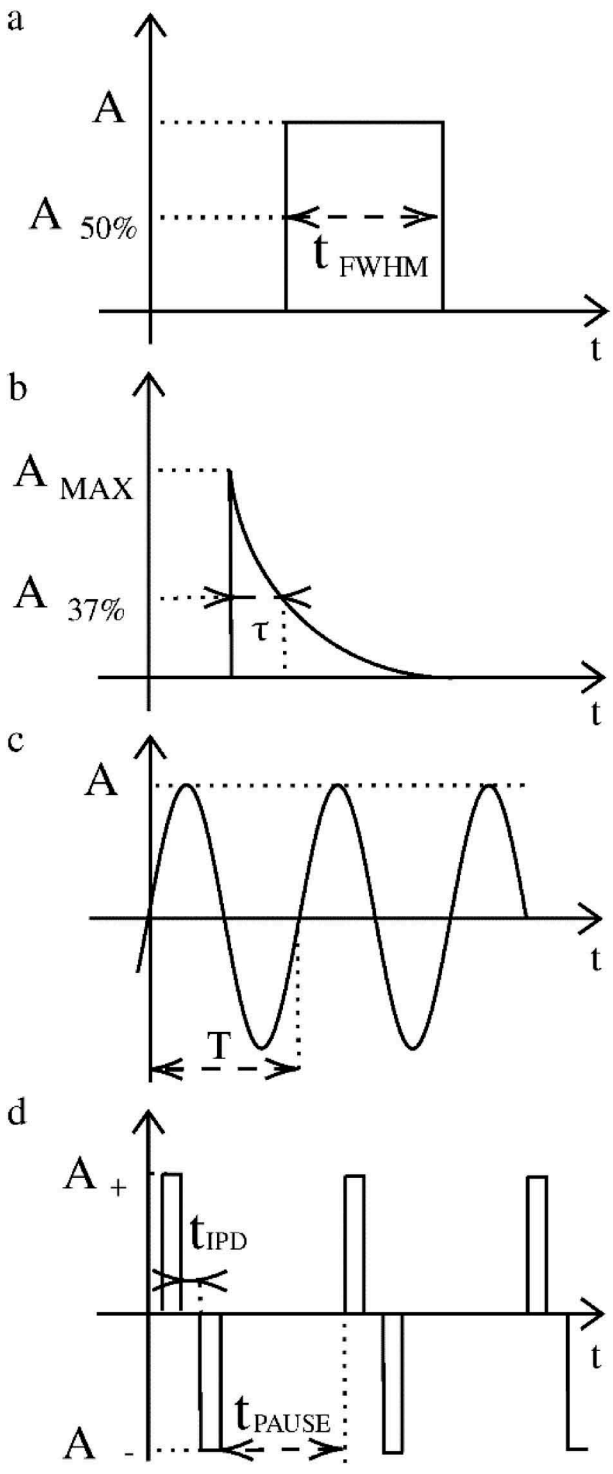

Figure 3. Typical waveforms applied in skin electroporation treatments. (a) Square wave pulses with durations (t_{FWHM} – the time of full width at half maximum) from a few nanoseconds to several hundred milliseconds and pulse amplitudes (A) from a few tens to several hundred volts. (b) Exponential pulses with the time constant (τ) from 1 ms to a few hundred milliseconds. c) Sinusoidal waveform, with different periods (T) and amplitudes (A). d) Bipolar pulses with varying pulse durations (t_{FWHM}), delays between half periods (i.e. inter-pulse delays t_{IPD}) and delays between bipolar pulses (t_{PAUSE}).

comparison of different devices and reproducibility of experiments are therefore difficult if not impossible. We thus suggest making use of mathematical models of skin electroporation, which can be used to calculate and predict the differences among various protocols observed in the literature by taking into account different pulse parameters, electrodes, and skin structure. Choosing the optimal pulsing protocol and electrode configuration could increase treatments' efficiency, and exploit its full potential with respect to other, currently leading technologies in the field of transdermal drug delivery and gene therapy.

3.1. The models of skin electroporation

Models offer an insight into the mechanisms of skin electroporation, and we firmly believe in their significant contribution to its better use and further development by elucidating the steps in skin electroporation, the importance of different parameters and decreasing the number of needed *in vitro* and *in vivo* experiments. In models, the number and thickness of the layers, their dielectric, thermal, and transport properties can easily be changed and various pulse combinations tested without expensive, ethically questionable and time-consuming experimental work. Regardless of the desired skin electroporation application (electrochemotherapy, irreversible electroporation, gene electrotransfer), tissue type (normal skin or tumor), and body part, taking into account different dielectric properties, modeling enables the description and prediction of electric field distribution, tissue heating, DNA distribution, etc., and thus the outcome of the chosen application, as well as translation from *in vitro* to *in vivo* and to human skin or optimizing/designing electrodes and even defining load for pulse generator.

From models, we can observe that small differences in the skin structure and parameters of applied pulses have a large influence on the treatment outcome. It was theoretically shown that small differences in the thickness of the *stratum corneum* (SC) affected the size of the LTRs and the electrophoretic force, pushing molecules across the skin [32] and thus, the treatment efficacy. Additionally, we conducted a parametric study of a model of electroporation of skin patch as a proof-of-principle of how small changes in the skin or pulse properties can have a large effect on the treatment outcome. We modeled the noninvasive multi-electrode array with 570 V pulses applied between every two pins [11] (Figure 4). The initial parameters of the model (skin geometry and dielectric properties) were obtained from [6], and electroporation was calculated sequentially as in [91]. The obtained results were our baseline. We modeled (1) the effect of skin hydration and/or age by varying the initial SC conductivity, (2) increase in SC conductivity after electroporation, (3) the body part by varying the thickness of SC, (4) different pulse generator by varying the applied voltage and (5) electric conductivity of all layers (Table 3). We calculated the reversibly and irreversibly electroporated volume and normalized them to the baseline. We observed that only a two-fold difference in initial or electroporated SC conductivity, which can easily be expected in experiments, increased the electroporated volume up to 30%. Increasing the thickness of the SC to 40 μm (from 20 μm) increased the reversibly electroporated volume but surprisingly, not the irreversibly electroporated. Decreasing the voltage by half did not decrease the electroporated volumes by half as one could expect but to 45% (reversible)

Table 2. Pulse generators usually used in studies on skin electroporation. For each manufacturer, we list the existing pulse generators, the intended use, the waveform type, pulse number, amplitude, and duration, reference to a study or manufacturer's webpage and our expert opinion on the generator. As we did not have access to all the listed generators, some were not tested or older versions than listed were tested as specified under each expert opinion.

Manufacturer	Pulse generator	Used for	Waveform type	Pulse number	Pulse amplitude	Pulse duration	Reference	Expert opinion
Amico	Mezoforte Duo[a]	Cosmetics (mesotherapy)	NA	NA	NA	NA	[77]	The device was tested in our laboratory. It delivers standard low-voltage radiofrequency (RF) electric pulses to the skin. There are three different applicators available for the device. One for RF and two for mesotherapy – "electroporation". The device allows us to set up the duration of the therapy (minutes), change the programs (face, body, hand …) and change the intensity of the therapy (low, standard, high). It generates a sinusoidal output signal of 833 kHz. The sinusoidal pulses are generated in bursts (modulated). The duration of the bursts is 4.3 ms for the mesotherapy and 30 ms for the RF therapy. The delays between the bursts are 8.5 ms for mesotherapy and 17 ms for RF therapy. There are no changes in the output signal characteristics due to the change in the selected program. Only the suggested time of treatment varies with programs. The highest voltages achieved measured at the therapeutic surface are 25 V_{pp} (peak-to-peak) for meso applicator and 32 V_{pp} for RF applicator.
BTX HARVARD APPARATUS	AgilePulse In Vivo System (formerly the DermaVax device)	Skin rejuvenation, transdermal drug delivery	NA	3 groups of pulses: from 1–10 pulses in each group	(50–1000) V	(0.050–10) ms	[manufacturer][b]	Gemini devices 'sense' the load each time before the pulse delivery. We tested Gemini X2 and a single 15 V, 45 ms long pre-pulse is delivered approximately 4 seconds before the pre-set sequence. The maximal current is not defined, but for Gemini X2 we determined it to be close to 11 A. Therefore when working with more conductive loads (< 80 Ω), the device is actually not able to deliver the whole range of pulse amplitudes defined in the specifications. We tested also the ECM 830, This device was also tested also on 50 Ω and 100 Ω resistors. From the measurements, we assume that this electroporator cannot deliver more than 1500 V to 100 Ω load and is almost not to be used for 50 Ω loads and lower. Overall a reliable manufacturer, with high quality products. The main drawback are the pre-pulse and missing visualization of delivered pulses.
	ECM 830[a]		Square wave	1–99	HV: (505–3000) V LV: (5–500) V	HV: (10–600) µs LV: (10–999) µs; (1–999) ms; (1–10) s		
	ECM 630		Exponential decay wave	1–99	HV: (50–2500) V LV: (10–500) V	10 µs–10 s		
	Gemini SC2		Square waves and exponential decay waves	LV: 1–10 HV: 1 − 2 Exponential decay: 1	(10–3000) V	50 µs–100 ms		
	Gemini X[2a]		Square waves and exponential decay waves	Square wave: LV mode:-1-120 (10 per sample) HV mode:1-36 (3 per sample) Exponential decay- 1-12 (R internal <100 ohms) and 1–24 (R internal > 100 ohm)	(5–3000) V	10 µs–1 s		
Cyto Pulse Sciences	Easy Vax	Gene electrotransfer	NA	NA	NA	NA	[10,78]	The device is not commercially available anymore.
DermaWave Company, USA and BTL Industries	DermaWave	Cosmetics (mesotherapy)	NA	NA	NA	NA	[[79],chap.12]	Cannot comment, due to the lack of the output pulse specification.

(*Continued*)

Table 2. (Continued).

Manufacturer	Pulse generator	Used for	Waveform type	Pulse number	Pulse amplitude	Pulse duration	Reference	Expert opinion
Equibio	Easyject Plus	Transdermal drug delivery	NA	NA	NA	NA	[80]	The data about the device could not be found, on the internet, maybe they were available in the past, it looks like device is not available anymore.
Genetronics Biomedical	MedPulser	Electrochemotherapy	Square wave	NA	~ 200 V/cm	60 ms	[10,81]	The device in not available anymore, Inovio upgraded it to CELLECTRA.
Ichor Medical Systems	TriGrid™Delivery System	Gene electrotransfer	NA	NA	NA	NA	[14]	The manufacturer chose not to provide the pulse characteristics, due to being a subject of their intellectual property.
IGEA	Cliniporator EPS02[a]	Electrochemotherapy, gene electrotransfer, transdermal drug delivery	Square wave	LV: 1–10 HV: 1 – 10	LV: (20–200) V HV: (100–1000) V	LV: (1–200) ms HV: (50–1000) µs	[manufacturer][b]	Both devices are clinical electroporators, classified as a medical device with a CE mark. Their operation is reliable and of good quality. We evaluated both and were pleased with their operation. They can however only be used in combination with manufacturers' electrodes, which are quite expensive and for a single-use. The only drawback is too low sampling frequency of the built-in measuring system.
	Cliniporator VITAE[a]			HV: 4 + 4 (polarity exchange); 4 – 8	HV: (500–3000) V	100 µs		
INOVIO	CELLECTRA®: 5PSP 2000 – 5P 2000 – 3P	Gene electrotransfer	Square wave	3 3 2 sets of 2 pulses	max 200 V	52 ms	[manufacturer][b]	The data presented in the table were provided directly from the manufacturer. However, the parameters might have changed over time. The device is built in the shape of an injection gun and it is used *in vivo* intramuscularly. It is not yet FDA approved but clinical trials are in full swing.
Jouan	Societe Jouan[a]		Square wave	one or continues	0 – 1500 V	5 µs – 24 ms	[94]	The device is used in our laboratory, pulse shape is not exactly square wave and measuring is necessary because output pulses do not match the preset characteristics. It is not produced anymore and quite rare.
Leroy BIOTECH	ELECTROvet S13	Gene electrotransfer	Square wave	1–10 000	0–1350 V	5–5000 µs	[manufacturer][b]	B10 and S13 have 25 A current limitation and B20 and EZ have only 10 A current limitation. The device evaluates the impedance of the connected load and disables the pulse delivery in case of a too conductive load (the user is properly informed about the fault). Thus, all parameter ranges are not available in all situations. The B10 device was evaluated in our laboratory and some problems were detected at low voltages (under 100 V) and short pulse durations (e.g. 10 µs). Overall a reliable manufacturer, with quality products.
	ELECTROvet EZ			1–10000	0–1500 V	5–5000 µs		
	ELECTRO cell B10[a]		Square wave bipolar	1–10000	0–1000 V	5–5000 µs		
	ELECTRO cell S20		Square wave	1–10000	0–2000 V	5–5000 µs		
Microlab International	Acthyderm[a]	Cosmetics (mesotherapy)	NA	NA	NA	NA	[82]	Cannot comment, due to the lack of the output pulse specification. However, the older version was tested in our laboratory and actually delivered radiofrequency waves to the skin.
	Max-E48	Cosmetics (mesotherapy)	NA	NA	NA	NA	[83]	The data about the device could not be found on the internet, maybe they were available in the past, it appears the device is not produced anymore.
OncoSec Medical	IMMUNOPULSE™ IL-12	Gene electrotransfer	NA	NA	NA	NA	[10,14]	The device is used only in clinical trials; it seems is still under development. The manufacturer declined to provide pulse characteristics, claiming they are a subject of intellectual property.
UltraVolt	NeoPulse Rack-2-500-00230	Power supply unit	Power supply unit	Power supply unit	Power supply unit	Power supply unit	[63]	Not an actual electroporator but a high-voltage power supply. For pulse generation an additional pulse forming circuit is required.

[a]Evaluated in our laboratory; [b]Technical specifications approved by the manufacturer; NA, not available; LV, low-voltage pulses, HV, high-voltage pulse.

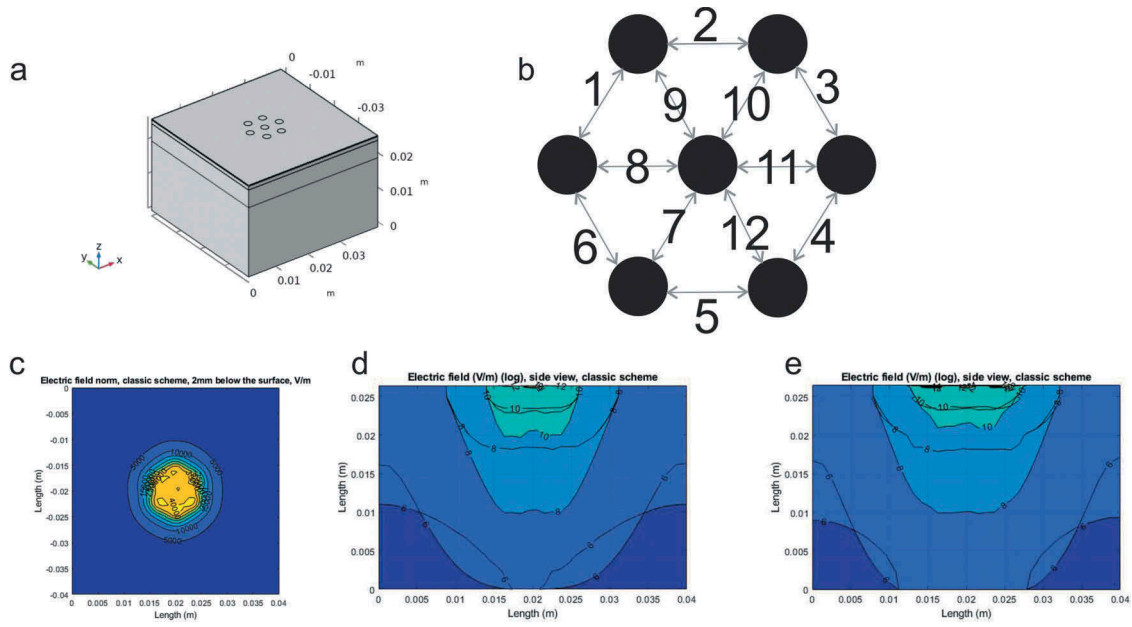

Figure 4. A numerical model of skin electroporation, which we calculated in the scope of this paper. (a) The geometry of the layered skin with the circles marking the pins of the multi-electrode array on the surface of the *stratum corneum*. (b) Classical pulse application scheme usually used in gene electrotransfer studies. Numbers mark the order of pulses in our simulation. (c) The electric field distribution 2 mm below the skin surface (in the hypodermis) when 570 V is applied when following the pulse application scheme on B. (d) and (e) show the side view of the logarithm of electric field distribution when the thickness of the SC is (d) 20 µm or (e) 40 µm. We can see that a minor change in the thickness of one layer significantly influences the distribution of electric field even 2 cm below the surface in the muscle layer.

Table 3. The volume of reversibly and irreversibly electroporated skin in the layered skin model as calculated in the scope of this paper. First, we calculated the volume of reversibly and irreversibly electroporated volume and then varied the parameters of the model. For each change in parameters, we normalized the results to the results with initial values of the parameters (the baseline).

Change of parameters Normalized volume	Reversibly electroporated volume	Irreversibly electroporated volume
Baseline	100%	100%
Increased SC conductivity (2-times)	124% of the baseline	122% of the baseline
Increased electroporated SC conductivity	123% of the baseline	130% of the baseline
Increased SC thickness	120% of the baseline	98% of the baseline
Decreased voltage (50% of the initial one)	45% of the baseline	17% of the baseline
Changed threshold of electroporation (increased 2-times)	98% of the baseline	91% of the baseline
Increased conductivity of all layers (2-times)	100% of the baseline	124% of the baseline

SC = *stratum corneum*

or only 15% (irreversible) of the baseline. We can thus see that already small differences in the skin structure, which are foreseeable in experiments across different animal species or even in the same subject, could be responsible for poor reproducibility and translation in applying the *in vivo* results from animal studies to human studies and that modeling of skin electroporation is useful.

Although the mathematical models of skin electroporation are promising, they do come with their drawbacks. Currently, the largest drawback is the lack of reliable parameter values [6]. The values of the parameters used in the models of human skin, come from porcine skin [25], properties of keratinous fibers [24,25], *ex vivo* human cadavers frozen for different time [26,28,92], geometry of skin cells [6], are deduced from physical constants and/or are based on only few measurements. Also, the place of the measurement is not always provided, and the measuring protocol not well described. The values of some parameters are only estimated, especially the dielectric properties of different layers after electroporation. Moreover, the dielectric properties of the skin are anisotropic, which is rarely considered in models. Also, all models should be validated by actual measurement. The models of skin electroporation were mostly constructed for human skin, and some were also validated on it [26,44], however, others were validated on rat [27], porcine [6], mice skin [40], with analytical solutions [24,25,33,36] or were not validated [32]. It was already shown that skin layers differ vastly in their dielectric properties, which significantly affects the results of calculations [25]. However, some models still model skin as a single layer of homogeneous properties [50,93,94], which can lead to erroneous results.

In future, new models should be developed and existing ones improved. More good-quality measurements of properties of skin should be performed and made available. The modeling focus should go in the direction of mechanistic multi-scale modeling and linking the phenomena at different levels – molecular, single-cell, organ, and tissue [95]. The successfully permeabilized/transfected region should be predicted by taking into account the LTR and pore formation, thermal, chemical, and electric tissue damage, the amount of the drug/DNA in contact with the cells [51,52], and other parameters, deemed to be relevant for skin electroporation. Better models will enable better treatment outcome prediction and more controlled treatment, which will pave the way

to improved efficacy, facilitate translation, and enable routine use of skin electroporation in the clinical setting.

3.2. The dosimetry

In skin electroporation, many different pulse generators were used (listed in Table 2). Unfortunately, in most cases, the delivery of electroporation pulses was not properly monitored [96]. Measurement of electroporation pulses is crucial to determine and control their quality and delivery. Current through the electrodes should always be measured to make sure that the pulses were applied to the biological load. Additionally, pulse generators cannot always be trusted due to poor regulation and lack of standardization. Because of large variety of biological loads with significantly different dielectric properties (also due to different electrode geometries used), pulse generators are not always able to deliver what they promise. In case of loads with low impedance and use of pulses parameters in the higher operation range, the delivered pulses can have a significant voltage drop due to the insufficient energy storage. On the other hand, the voltage amplitude can be limited because of current limitations. Some devices warn the user about the improper operation while others not. Another problem is that pulse parameters of some devices cannot be changed and/or pre-programmed setups without known pulse parameters are used. Consequently, studies often lack the information on pulse specifications (shape, duration, number, voltage, repetition frequency) and thus cannot be reproduced or compared with other studies.

Applied pulses are of different shapes, durations, voltages with their spectral energy contained within different parts of the spectrum. Dielectric properties of tissues are frequency-dependent [97], which influences the electric field distribution across the skin layers and consequently electroporation efficiency [98]. Currently, there are no agreed standard operating procedure pulse parameters for skin electroporation, except for the treatment of cutaneous and subcutaneous tumors [21]. More studies should be performed, determining the most efficient waveform(s) for skin electroporation. Although there is a significant number of pulse generators available on the market, versatile generators should still be developed, for example, for applying bursts of short bipolar pulses, i.e. the high-frequency irreversible electroporation (HF-IRE) pulses [99] to reduce pain and muscle contraction during skin electroporation [14]. The generators, producing the optimal waveforms being applied to the skin by optimal electrode configurations should be reliable, simple to use, safe, with available technical specifications, feedback quality measuring system and, when used in the clinics, should comply with the standards for medical devices [100,101].

Various electrode configurations are used in the skin electroporation studies, and the description on electrode geometry is often poor or lacking which additionally renders the dosimetry inaccessible and comparing difficult if not impossible. Moreover, in many studies, only the applied voltage is reported although it was shown that the electric field is the most important parameter influencing the efficiency of electroporation [102] and different electrode configurations cause significantly different electric field distributions [84]. The electrodes are of different materials (stainless steel, platinum, silver, silver chloride, brass, gold) which can cause different chemical reactions and metal release which also can affect the treatment outcome [103–105]. Interestingly, it was experimentally shown that gene transfection of skin cells [106,107], as well as electrochemotherapy of subcutaneous tumors [108], could also be achieved contactless with pulsed magnetic fields (PEMF) which could decrease the chemical contamination and facilitate the use of noninvasive techniques and is worth exploring in the future. Electric field distribution should be calculated and shown for each configuration separately enabling comparison of different electrode configurations.

Various pulse shapes, generators, and electrode configurations all contribute to vastly different dosimetry among the published studies, and the dosimetry is not always adequately reported. All this renders comparison of different studies difficult, if not impossible, especially if not all the details are presented. In electrochemotherapy, the pulse parameters and electrode configurations are now standardized to provide safe and efficient treatment for the patients [21]. A similar attempt should also be made in the field of other skin electroporation treatments. We thus ask the researchers to follow the instructions for reporting the dosimetry, as suggested in [85–87,100]. Using good-quality pulse generators together with controlled dosimetry, and predictive modeling should increase the efficiency of skin electroporation treatments, enable comparisons between treatments and simplify the translation into clinics.

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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