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# *In vivo* real-time monitoring system of electroporation mediated control of transdermal and topical drug delivery

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ABSTRACT

Electroporation (EP) is a physical method for the delivery of molecules into cells and tissues, including the skin. In this study, in order to control the degree of transdermal and topical drug delivery, EP at different amplitudes of electric pulses was evaluated. A new *in vivo* real-time monitoring system based on fluorescently labeled molecules was developed, for the quantification of transdermal and topical drug delivery. EP of the mouse skin was performed with new non-invasive multi-array electrodes, delivering different amplitudes of electric pulses ranging from 70 to 570 V, between the electrode pin pairs. Patches, soaked with 4 kDa fluorescein-isothiocyanate labeled dextran (FD), doxorubicin (DOX) or fentanyl (FEN), were applied to the skin before and after EP. The new monitoring system was developed based on the delivery of FD to and through the skin. FD relative quantity was determined with fluorescence microscopy imaging, in the treated region of the skin for topical delivery and in a segment of the mouse tail for transdermal delivery. The application of electric pulses for FD delivery resulted in enhanced transdermal delivery. Depending on the amplitude of electric pulses, it increased up to the amplitude of 360 V, and decreased at higher amplitudes (460 and 570 V). Topical delivery steadily enhanced with increasing the amplitude of the delivered electric pulses, being even higher than after tape stripping used as a positive control. The non-invasive monitoring of the delivery of DOX, a fluorescent chemotherapeutic drug, qualitatively and quantitatively confirmed the effects of EP at 360 and 570 V pulse amplitudes on topical and transdermal drug delivery. Delivery of FEN at 360 and 570 V pulse amplitudes verified the observed effects as obtained with FD and DOX, by the measured physiological responses of the mice as well as FEN plasma concentration. This study demonstrates that with the newly developed non-invasive multi-array electrodes and with the varying electric pulse amplitude, the amount of topical and transdermal drug delivery to the skin can be controlled. Furthermore, the newly developed monitoring system provides a tool for rapid real-time determination of both, transdermal and topical delivery, when the delivered molecule is fluorescent.



## 1. Introduction

Transdermal drug delivery offers an attractive non-invasive alternative to the conventional delivery methods, such as oral administration and injection. The main advantage of the delivery through skin is the possibility of molecules to enter the circulation, avoiding the metabolic processing of the delivered molecules in the liver. However, the stratum corneum acts as a limiting barrier, therefore only small lipophilic drugs have the ability to penetrate the skin at therapeutic rates by passive diffusion [1,2]. Conventional transdermal delivery systems, such as transdermal patches, enable controlled transdermal drug delivery, but are applicable only to small, potent and lipophilic solutes and the transport of drug across the skin is slow with lag times to reach steady-state fluxes in hours [2,3]. Therefore, to deliver larger molecules with therapeutic drug levels, many chemical and physical delivery methods were developed [4]. Many studies were focused on so-called active strategies such as sonophoresis [5–9], iontophoresis [10–12] and electroporation [1,13–17]. However, the existing monitoring systems lack the real-time monitoring of topical and transdermal drug delivery, as well as the ability of its quantification.

Electroporation (EP) is a physical method for the delivery of molecules into the cells and tissues [18,19]. Currently its biomedical applications are the delivery of (i) chemotherapeutics (electrochemotherapy) [20,21], (ii) naked plasmid DNA or RNA (gene electrotransfer) for tumor treatment and DNA vaccine [15,22–24] or (iii) drugs/DNA delivery in or across the skin [25–31]. EP mediated delivery of drugs and DNA

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to the skin has already been successfully performed in rodent, porcine and non-human primate skin models [16,29,32,33]. For the delivery to the skin several different electrode types were used, from plate, needle, needle free microelectrode array to multi-electrode array [13,34–39]. Depending on the molecules to be delivered, long (ms) pulses are needed for naked DNA delivery, while short (μs) pulses can be used for the smaller molecules like drugs. Depending on the electrode type, the delivery of molecules can be controlled by the number, amplitude, and duration of electric pulses [13,40–42]. Non-invasive electrodes are particularly interesting to localize the electrical field in the skin [16]. However, EP using non-invasive multi-array electrodes with spring loaded pins was not tested yet for the controlled transdermal and topical drug delivery.

Besides the delivery of molecules to target cells in tissues, the application of electric pulses also has vascular effects on normal and tumor blood vessels [43–46]. On the experimental tumors it was shown that the vascular effects of EP, *i.e.* vasoconstriction and increased permeability of blood vessels, are dependent on the number and especially the amplitude of electric pulses. Namely, the vascular effects were observed only after certain threshold amplitude was reached and its further increase prolonged them [47]. Recently it was shown that EP can indeed increase the permeability of tumor blood vessels and with the use of intravital microscopy it was also confirmed on normal blood vessels in skin [44], meaning that EP induces vasoconstriction of all exposed blood vessels as well as increases their permeability [46,48]. Therefore, when EP is used for the topical and transdermal drug delivery, the vascular effects of EP should be taken into account.

In this study we investigated the relationship between the transdermal and topical drug delivery, controlled by EP of the skin. For this purpose we used a new non-invasive multi-array electrodes and different high-voltage EP parameters. In order to monitor the topical and transdermal delivery we developed a real-time monitoring system, based on fluorescently labeled molecules, that can qualitatively and quantitatively follow the extent of topical and transdermal delivery. Additionally, the established monitoring system was, as a proof of principle, also used for the topical and transdermal doxorubicin delivery. In order to verify the effect of pulse amplitude on transdermal and topical delivery as well as to verify the monitoring system, standard fentanyl assays [49] were also performed.

## 2. Materials and methods

### 2.1. Reagents and drugs

The 4 kDa fluorescein-isothyocianate (FITC) labeled dextran (FD) (Sigma-Aldrich, St. Louis, MO, USA) was resuspended in phosphate buffered saline (PBS). In order, to remove any free FITC or low-molecular weight contaminants, the FD was washed two times for 2 h through 2 kDa ultrafiltration spin column (Vivaspin, Sartorius Stedim Biotech, Goettingen, Germany). The component with high molecular weight was afterwards resuspended in PBS to a final concentration of 37.5 mg/mL. In addition, Doxorubicin hydrochloride (DOX) obtained from Teva (Teva Pharmaceutical Industries Ltd, Pharmachemie B.W., Haarlem, Netherlands) and Fentanyl (FEN) purchased from Chiesi PharmaceuticalGmbH (Torrex; Chiesi Pharmaceutical GmbH, Wien, Austria) were used as model drugs to study the extent of transport into and through mouse skin into systemic circulation. The concentration of stock FD solution was 37.5 mg/mL [46,48] and concentration of stock FEN solution was 50 μg/mL. Epirubicin hydrochloride, which was used as an internal standard for chemical analysis, was purchased at Actavis (Episinidan; Actavis, Hafnarfjordur, Iceland). LC–MS grade acetonitrile and water used as mobile phases were purchased at J.T. Baker (Phillipsburg, NJ, USA), whereas the mobile phase additive formic acid (50%) was obtained from Sigma-Aldrich. All solvents and chemicals employed in sample preparation (methanol, water and sodium acetate) were of analytical grade purity.

### 2.2. Animals and skin preparation

In the experiments 10–12-week old female BALB/c mice (Harlan Laboratories, Udine, Italy) weighing between 20 to 25 g were used. Mice were kept under specific pathogen-free condition at a constant room temperature and humidity and at 12 h light/dark cycle. Food and water were provided *ad libitum*. All experiments were conducted in accordance with the guidelines for animal experiments of the EU directive and permission from the Ministry of Agriculture and the Environment of the Republic of Slovenia (permission 34401-1/2012/4). For each experimental condition 3–6 mice were randomly assigned. Mice were shaved on the flank region and any remaining hair was removed with depilatory cream (Vitaskin; Krka, Novo mesto, Slovenia). Moreover, to reduce the autofluorescence, the tail of mice was also depilated before the experiments with FD and DOX where fluorescence microscopy imaging was employed.

### 2.3. Drug application

In all experiments a circular patch with the diameter of ~1 cm (Tosama d.o.o., Domzale, Slovenia) served as a reservoir for molecules or drug application. In case of EP the patch soaked with FD (110 μL of 37.5 mg/mL), DOX (100 μL in dose of 10 mg/kg) or FEN (~120 μL in dose of 0.3 mg/kg) was applied on the depilated mouse flank region for 5 min, which was first rinsed with a piece of wet cotton. The patch was removed during the EP and afterwards it was reapplied and left on the skin for 1 h. Subcutaneous injection of the equal quantity of FD, DOX or FEN, as applied on the patches, was referred as 100% transdermal delivery. The tape stripping method [50] served as a positive control. An adhesive tape-strip was placed and gently pressured onto the depilated area of mouse skin, by which a good contact with skin was ensured. Subsequently, the tape was removed with sharp upward movement. These two steps were repeated 15–20 times, which enabled the removal of the stratum corneum layer.

### 2.4. Electrodes and EP parameters

The non-invasive multi-array electrodes, consisting of 7 spring loaded pins arranged on hexagonal mesh and spaced 3.5 mm between each other, were provided by Iskra Medical (Podnart, Slovenia) (specifics are provided in the Supplementary material and methods). Electrodes were connected to the CLINIPORATOR™ (IGEA s.r.l., Carpi, Italy). Different short high voltage square wave pulses (amplitude between 70 and 570 V) with duration of 100 μs were used for the transdermal delivery of FD. A total of 24 electric pulses (2 electric pulses between each electrode pair) were delivered during the treatment. For transdermal delivery of DOX and FEN only the amplitudes 360 V and 570 V were applied.

### 2.5. Fluorescence microscopy and image acquisition

Fluorescence microscopy was carried out with a Zeiss SteREO Lumar.V12 (Zeiss, Jena, Germany) fluorescence stereomicroscope equipped with an MRc.5 digital camera (Zeiss). Animals were initially anesthetized with inhalation anesthesia in the induction chamber (Isofluoran; Nicholas Piramal India, London, UK) and placed under the microscope, with their snout in the inhalation tube to remain anesthetized during the experiment. Furthermore, the tail was fixed with adhesive tape (Micropore, 3M Health Care, Neuss, Germany) on each side outside the field of view of the microscope to prevent its movement during the observation. To obtain 48-bit RGB images of emitted fluorescence light the appropriate filters (FD (excitation: 470/40 nm; emission: 525/50 nm), DOX (excitation: 470/40 nm; emission: LP 515 nm)) were used. A time series of images were acquired with the following timeline; firstly, one image was acquired before the application of the patch and another 5 min later, when the patch was removed for EP; afterwards,