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Non invasive contact electrodes for *in vivo* localized cutaneous electropulsation and associated drug and nucleic acid delivery

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ABSTRACT

For an effective tissue controlled electropermeabilization as requested for electrochemotherapy and electrogenotherapy, it is very important to have informations about the electric field distribution provided by a defined set of electrodes. Computer simulations using the finite element models approach predicted the associated field distributions and currents. Phantoms made of gels with well-defined electrical conductance were used to measure the current responses of a new electrode geometry (wires), A good agreement between the measured and predicted currents was observed supporting the validity of the prediction for the field distribution.

Field distribution was observed to be very localized and highly homogeneous with the new concept of contact wire electrodes. They allowed to focus the field effect along the surface of the tissue to induce a controlled release of drugs or plasmids. Non invasive (contact) electrodes can be moved rapidly on the body and avoid puncturing the skin and the tissue. They can be used for large surface effects, to treat the skin and subcutaneous tumors. The use of contact electrodes after drug or DNA intradermal injection were validated by clinical treatment of large surface skin tumors and by *in vivo* imaging of permeabilization or of gene expression.

1. Introduction

Cell treatment with high intensity electric field pulses provokes a change in the membrane structure leading to a loss of its barrier function, a phenomenon called electropermeabilization or "electroporation" [11,23]. By a proper choice of the parameters of the applied electric field (i.e. amplitude, pulse duration and number of pulses), this change in the membrane permeability can be reversible preserving cell viability or irreversible leading to cell death. Electropermeabilization brought the technical possibility to introduce (load) exogenous compounds (drugs, plasmids) into cells. It is now used as a very efficient way for drug, oligonucleotide, antibody and plasmid delivery *in vitro* and *in vivo* for clinical applications [11,23].

Electrotransfer of plasmids has been performed *in vivo* on several tissue types including skin, liver, tumor, muscle, brain, testis and spleen [2,5,15,18,24,29,34]. Many studies have now shown that plasmid electrotransfer can lead to a long-lasting therapeutic effect in various pathologies, such as cancer, blood disease, or muscle ischemia. These have been reviewed in [3,6,14,20] Clinical developments are obtained under optimized electrical conditions [35]. The local field strength appears as the critical parameter for *in vivo* studies [8,12,28]. Its value must be larger than a threshold to trigger the permeabilization process but below a value inducing irreversible damages. These biological effects are under control of the pulse duration. Therefore application of square wave pulses appears the most suitable. Field effects on cells in tissue are rather similar from what is obtained for cells in suspension. They nevertheless depend on the cell density [25] and on the different shapes of cells [36]. The local field strength remains the critical parameter [35]. It is the field distribution in the tissue which is important [1,21,20].

While field distribution is homogeneous when diluted cells are pulsed in suspension between parallel plate electrodes, the problem is much more complex in tissue. As the field results from a voltage applied between two electrodes, the electrode configuration is clearly controlling the field distribution and therefore the effective uptake. Various electrode configurations for therapeutic purposes are available such as parallel plates, wire and contact plate electrodes [9] as well as needle electrodes and needle arrays [13,19,27,37].

Electrode configuration influences the electric field distribution in tissue. Needles are popular but mechanically invasive and the associated field distribution is very heterogeneous [4,17,22]. They were shown to induce local burning due to their very high current

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density close to the electrode surface. Joule heating can therefore be very high [26]. Parallel plates are rather popular when their use is possible as when pulsing mouse thigh muscle [7] or when skin pinching is possible [5,29]. Meander electrodes affect the stratum corneum (SC) and support transdermal delivery [17].

It is therefore necessary to choose appropriate electrode configuration for the particular target tissue before it is exposed to therapy. In order to obtain such information, electric field distribution in tissue has to be computed in advance by means of modeling. Due to tissue complexity, analytical solution of such problem is almost impossible. Therefore in most cases numerical modeling techniques are used. Mostly finite element method and finite difference methods are applied [31]. Both numerical methods have been successfully used and also validated by comparison of computed measured electric field distribution and associated consequences in tissues [32]. Finite element model validation by computed reaction current was used only very recently [32]. Current measurement is much faster than imaging method, which can be used to obtain electric field distribution in tissue. Therefore we selected the finite element model validation with experimental current measurements as a fast method of model validation.

But it is also well known that numerical methods are computationally demanding. Therefore, it is required to search for simplifications in modeling process which can decrease computational efforts and at the same time preserve the accuracy of the result, i.e. electric field distribution or current. Such simplifications can be performed either on the description of electrodes or on the physical properties of tissue. In this work, we have assumed simple shapes for electrodes (polygonal rather than cylindrical). Tissues (in our case: dermis and epidermis, the SC being by passed within a few microseconds after the pulse onset) were assumed to have a homogeneous conductivity and soft gels prepared in saline buffers can be used as phantoms. This is indeed in fair agreement with ex vivo direct observations where large domains with homogeneous conductance are present in tumors [39].

Subcutaneous electropulsation is known to be relevant of clinical applications (subcutaneous tumors) and for genotherapy (DNA vaccines). It was predicted that contact wire electrodes should provide targeting of the field effect under the skin. Contact wire electrodes have been shown to be very convenient when large tissue surfaces (several square centimeters) must be treated due to the ease of their use [16,30].The field distribution from the simulation was validated by comparing the current values given by the simulation and their experimentally measured value (Fig. 1). Field lines were definitively proved to be focused along the skin. These physical predictions and conclusions were checked *in vivo*. As a conclusion, contact wire electrodes offer an approach, where electropulsation *in vivo* can be efficiently, easily and safely performed at the cutaneous level.

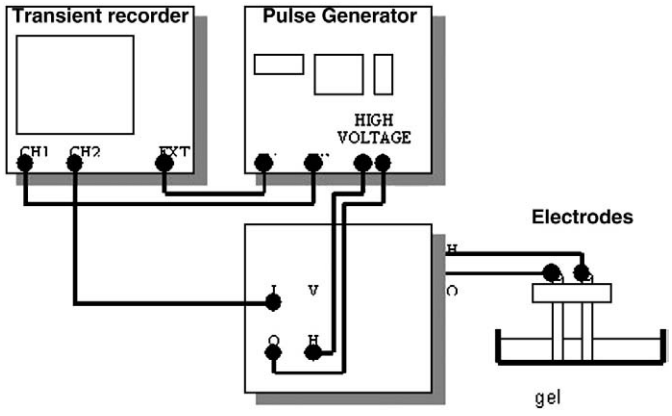

Fig. 1. Electrical set up. The high voltage is applied between the two electrodes in contact with the gel. The voltage and the current are recorded on line, digitized and stored. The time course of their ratio gives access to the time change of the impedance of the gel during the pulse.

2. Materials and methods

2.1. Tissue phantom

The cutaneous tissue is an ohmic conductor as soon as the stratum corneum is electropermeabilized and as long as the tissue is not destroyed by the field. Tissue phantom was made of gelatin (2.4% w/v) in phosphate buffer (concentration 20 mM, pH = 7.4) and NaCl (concentration 150 mM), with electrical parameters and characteristics close to real tissue. This is actually a gel with some rigidity when cooled. Due to moisture of the gel a good electrical contact was attained with electrodes in direct contact with the gel. Fresh gel was prepared from a buffer before each experiment and its conductivity was measured (conductimeter Hanna HI8820N, Germany). Tissue phantom conductivity was 1.5 S/m.

The phantom tissue was prepared in a Petri dish of 35-mm diameter. The thickness of the gel was adjustable to 2, 4 or 6 mm, depending on the requirements of the specific experiment. The thickness of gel was controlled by pouring a given volume of the hot liquid gel in the dish.

The voltage pulse was obtained with a high voltage square wave pulse generator (CNRS Jouan PS 10, France) (Fig. 1). Voltage up to 1000 V can be delivered as long as the current was less than 8 A. On this system, if a current surge exceeds this limiting value (8 A), an automatic switch is triggered for safety reasons and the pulse is discontinued. A resistor *R* was inserted in series with the electrode array to be used to monitor the current. Both a fraction of the voltage pulse delivered by the generator and the voltage across the resistor *R* were digitized (8-bits resolution) and stored on line with a transient recorder (Data Lab DL 905, UK). The stored signals were observed on an oscilloscope or analyzed on a MacIntosh LCIII microcomputer (Apple, USA) by using an ADA4 interface with an Excel subroutine. The system was calibrated for the current by using an ohmic calibrated high power resistor in place of the electrode-gel set up. Applied voltage was in the range 100 V to 500 V, in increments of 100 V. The pulse length was 0.1 ms (to limit Joule heating). By plotting *U*/*I* ratio during the pulse application, material conductivity was observed to be constant. A linear response of the system was observed for increasing values of the applied voltage (up to 1000 V). In some experiments, lower applied voltages (0 to 100 V) were studied with increments of 25 V. (Fig. 1)

Reproducibility of replicates accuracy in each experiment was high. It was the reason to assume three replicates per experiment were sufficient. Based on the fact that replicate results in all experiments were very similar we can assume that random measurement error was negligible.

2.2. Electrodes

Contact wires were two parallel stainless steel rods with a diameter of 1 mm, a length from 10 to 20-mm at a distance ranging from 4- to 9-mm for the different models (Figs. 2–5). Their penetration in the gel (or the extent of their contact with the skin) can be adjusted by exerting different pressures. A conductive gel was used to improve the contact with the skin in the *in vivo* experiments [39].

2.3. Simulation: finite element method

A three-dimensional finite element model of a gel in Petri dish with contact electrodes was designed using software package Emas produced by ANSOFT Corporation.

The geometry under study was moderately complex, involving few physical objects (gel, electrodes) with specific geometrical and