In vivo assessment of skin impedance recovery after low-voltage pulses electroporation

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Abstract- The aim of this work is to measure the skin impedance recovery after electroporation by using low-voltage pulse protocol on human subject. Before and after electroporation, based on square wave pulses of 20-50 V, 5 µs, for 15-20 minutes, Electrical Impedance Spectroscopy (EIS) measurements has been performed, in the frequency range 0.5-10 kHz. Preliminary experimental results show a significant decrease in skin impedance amplitude after treatment. This result turns out to be useful in a measurement procedure aimed at determining the amount of drug actually transferred in electroporation-based therapy.

I. Introduction

Historically, electroporation was defined as a short-duration high-voltage pulses applied to the skin, in order to enhance transdermal drug delivery by several orders of magnitude [1-5], and to permeabilize transiently cells in tissue [6-10]. Furthermore, electroporation of cell membranes is a well-known phenomenon, extensively used for DNA transfection in biological laboratories [7-8] and in electrochemotherapy.
Actually, tissue electroporation by short-duration, small-amplitude pulses (< 50 V) is under investigation for biomedical engineering applications of medical therapy aimed at cancer treatment, gene therapy, and transdermal drug delivery [11]. In particular, the dramatic and reversible increase in skin permeability caused by electroporation allows drugs to be delivered transdermally at significantly enhanced rates [5]. Actually, the critical point is to quantify the drug amount delivered into the skin after treatment.
This problem has been faced by measuring the change of skin electrical proprieties after treatment due to the drug injection, and in particular, by means of Electrical Impedance Spectroscopy (EIS) [12]. Nevertheless, the variation of skin electrical proprieties after treatment can be caused both by: (i) the presence of drug delivered into the skin; and (ii) the transient of skin impedance due to electroporation [13].
A relationship between drug delivered into the skin and impedance spectrum can be determined by investigating the transient of impedance spectra due to electroporation. Previous works have been assessed the skin impedance recovery after high-voltage pulse protocol on rabbit [13]. However, the specific recovery of human skin impedance in vivo after electroporation has not been investigated comprehensively on human subjects.
In this paper, the skin impedance recovery after electroporation by using low-voltage pulse protocol on human subject is analyzed. After a background on electroporation, the analysis procedure is illustrated and the preliminary in-vivo experiments, using a low-voltage pulse protocol suitable for human experiments, are reported.

II. Background

Electrical pulses, causing the trans-membrane voltage level of fluid lipid bi-layer membranes to reach at least $V_m \approx 0.2$ V (usually also 0.5–1.0 V), are hypothesized to create primary membrane "pores" with a minimum radius of ~1 nm [11]. Transport of small ions such as Na^+ and Cl^- through a dynamic pore population discharges the membrane even while an external pulse tends to increase V_m.
Molecular transport through primary pores, as well as pores enlarged by secondary processes, provides the basis for transporting molecules into and outside from biological cells. Cell electroporation is used in vitro mainly for transfection by DNA introduction, but many other interventions are possible, including microbial killing [11]. Ex vivo electroporation provides manipulation of cells to be

reintroduced into the body at therapeutic aims. In vivo electroporation of tissues enhances molecular transport through tissues and into their constitutive cells. Tissue electroporation, by longer and large pulses, is involved in electrocution injury [11].
Tissue electroporation by shorter and small pulses (< 50 V) is under investigation for biomedical engineering applications of medical therapy aimed at cancer treatment, gene therapy, and transdermal drug delivery [11]. It involves a complex barrier, containing high electrical resistance, multi-lamellar lipid bi-layer membranes, and a tough, electrically invisible protein matrix.
Nevertheless, recovery time of skin properties after small pulses electroporation has not been investigated extensively, especially on human subjects. In particular, the mechanisms correlated to skin impedance decrease due to electroporation were investigated [11]-[14], at 1 Hz, after electroporation, by using voltage pulses too high for human application (voltage pulse of both 1000 V and 335 V, at 100 µs and 5 ms, respectively) were analysed [13]. In recent works, experimental test showed that skin impedance recovers its previous-electroporation value after 4-6 hours.

III. The proposal

In this work, a preliminary analysis of the skin impedance recovery after electroporation is presented in order to define a measurement procedure aimed at determining the amount of drug actually delivered in an electroporation-based therapy. In particular, the analysis aims at discerning the after-treatment variation of skin impedance spectra caused by electroporation from the effect of the drug injection.
With this aim, the proposed analysis is based on two main steps: (i) a low-voltage pulse protocol for in vivo tests (20-50 V, 5 µs square wave pulse) is applied to human subjects for 15-20 minutes, and (ii) after the treatment, the skin impedance in a large frequency range (from 500 Hz up to 10 kHz) is investigated by using EIS measurements.
EIS measurements are performed by using a digital-measurement method for low-invasive clinical diagnosis, in order to overcomes current resolution limits of biological electrical-impedance analysis by means of several polarization levels [15].
In particular, the impedance measurement was based on the Direct Measurement method: a sinusoidal current stimulus is injected by means of the A+/A– electrodes and acquired, the relative voltage drop is acquired by means of the V+/V– electrodes. Thus, the magnitude |Zx| of the system under test can be evaluated as :

$$Z_x = \frac{|V|}{|I|} e^{j(\delta_V - \delta_I)}$$

where the phase ? of the impedance can be evaluated as the phase difference between the acquired sinusoidal voltage and current signals.

IV. Experimental results

The measurement system is based mainly on [16]: (i) a notebook PC, in order to process data and realize a user interface software, (ii) a multifunction I/O analog-digital conversion board, PCMCI DAQCard-6062E (National Instrument™, Austin, Texas), plugged into a digital device, in order to generate the stimulus signal and acquire voltage and current signals with electrical insulation; (iii) a conditioning block interface, based on analog signal power amplifiers, and (iv) suitable cables and electrodes, in order to apply and gather electrical signals from the patient.
Particularly, the analog interface design (measurement) is specifically aimed at handling low-amplitude signals, thus, differential amplifiers were used to guarantee high noise immunity and common-mode rejection, necessary in low-frequency measurements on living biological systems. Electrodes are disposable silver/silver chloride disposable electrodes with adhesive and conductive gel for neurological application 15 x 20 mm from Spes Medica ®.
The entire equipment was qualified according to IEC safety requirements.
Experiments were carried out in a laboratory room with controlled temperature (20 C) and relative humidity (3.0 %).
Tests were carried out on harm region of two healthy volunteers (28-35 years old). The region under test was large about 10 cm^2.
An electroporation protocol based on square wave pulse of 25-30 V, 5 µs, was applied for 15 minutes, has been provided by Acthyderm™ system from Microlab srl, used for standard application in aesthetic medicine.

Before electroporation, EIS measurements were carried out in a frequency range from 500 Hz up to 10 kHz. Then, after electroporation, the same EIS measurements were carried out after 30 s and 2,3, 10, 15, 25, 60 minutes.

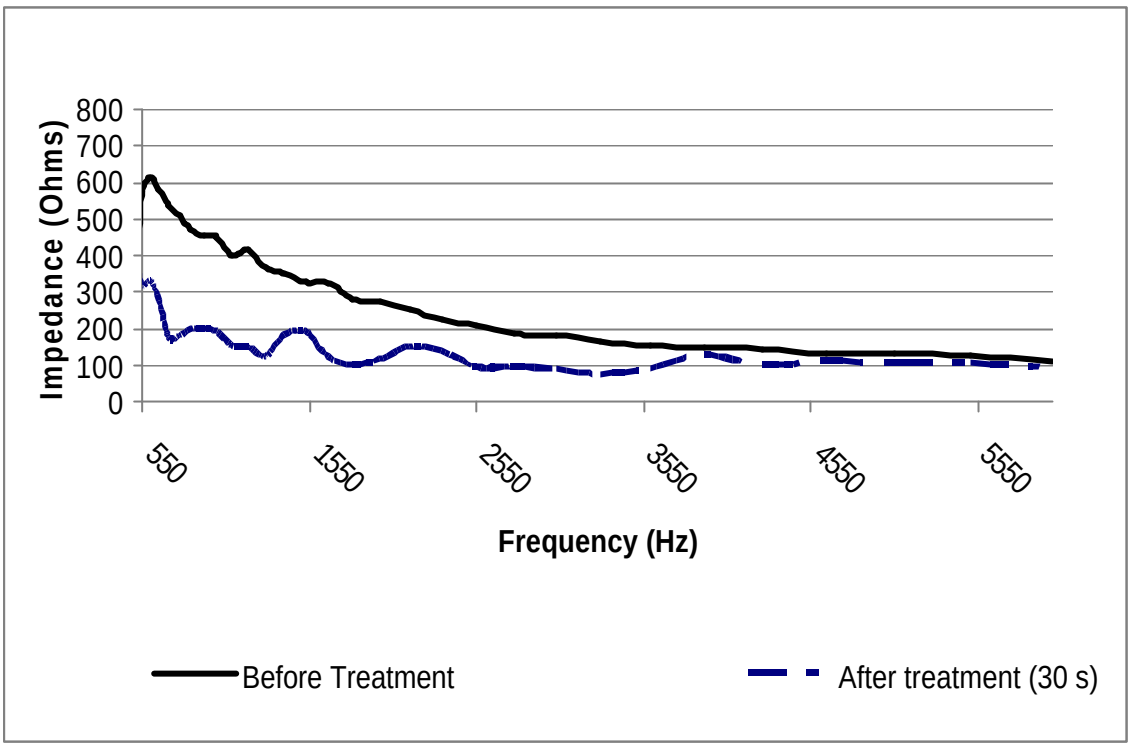

Figure 1: Impedance amplitude decrease after 30 s from treatment

In Fig. 1, the resulting amplitude impedance spectra is shown. After 30 s from the treatment, test shows a significant decrease of impedance spectra. This phenomen persists up to about 1 hour.
However, the decreasing of impedance spectra, such as shown in Fig 1, is more consistent in a frequency range up to 3.5-4 kHz.

IV. Conclusions

In this work, the variation in skin impedance after low-voltage pulse electroporation is analysed. The preliminary in-vivo experiments reported in this paper, using a low-voltage pulse protocol suitable for human experiments, show a significant decrease of skin impedance spectra after electroporation, in a large frequency range (0.5, 4 kHz).
This result turns out to be useful in a measurement procedure aimed at determining the amount of drug actually transferred in electroporation-based therapy.

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