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Detecting Electroporation by Assessing the Time Constants in the Exponential Response of Human Skin to Voltage Controlled Impulse Electrical Stimulation

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***Abstract*—We propose a new method for extracting the electrical properties of human skin based on the time constant analysis of its exponential response to impulse stimulation.**

As a result of this analysis an adjacent finding has arisen. We have found that stratum corneum electroporation can be detected using this analysis method. We have observed that a one time-constant model is appropriate for describing the electrical properties of human skin at low amplitude applied voltages (<30V), and a two time-constant model best describes skin electrical properties at higher amplitude applied voltages (>30V). Higher voltage amplitudes (>30V) have been proven to create pores in the skin's stratum corneum which offer a new, lower resistance, pathway for the passage of current through the skin.

Our data shows that when pores are formed in the stratum corneum they can be detected, in-vivo, due to the fact that a second time constant describes current flow through them.

I. Introduction

HUMAN skin electrical properties have been previously investigated using AC and DC methods [1]. Skin impedance values [2] and the values of the DC resistance and capacitance of human skin [3] have been reported. It is now clearly understood that human skin also has a non-linear behavior, which can be assessed both during AC [4] and DC [3, 5] stimulation.

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One of the interests of our research group is to assess the electrical properties of human skin during neuromuscular electrical stimulation (NMES). By the nature of the stimulus applied during NEMS (short, biphasic) no charge build-up will occur on the surface of the skin, thus the skin electrical properties can be analyzed using linear models. It is well understood that after the skin equivalent capacitor has fully charged, process that generally takes ~300μs [6], the stratum corneum high resistance is fully introduced in the circuit (Fig. 2A), and its value starts dropping due to charge build-up on the skin surface [3, 7]. This non-linear skin behavior will not occur in the case of voltage controlled NMES due to the fact that the voltage pulses applied are too short. It is also known that current travels through the skin appendages if the voltage drop across the skin is less then 30V, but if the voltage is increased, new pores will be formed in the skin's stratum corneum creating a new pathway for the current and drastically decreasing the stratum corneum resistance [6]. This shift between the electrical properties of human skin bellow and above 30V needs to be taken into account in the analysis of skin electrical properties during NMES.

We have chosen to analyze the electrical properties of human skin during voltage controlled NMES by trying to find the best equation that describes the exponential decay of the current response of the skin.

II. Methods and Models

All of the experiments conducted were approved by the NUI Galway Research Ethics Committee. The subjects also gave their informed consent prior to the commencement of the study.

Fourteen healthy subjects, seven male and seven female, aged 20 to 26, were investigated.

All subjects received NMES for a period of seven consecutive days, three times a day, using the Duo-STIM neuromuscular electrical stimulator [8]. One of the 30 minutes daily stimulation sessions took place in the laboratory where voltage and current measurements were performed at 5 minute intervals.

Single channel, biphasic, trapezoidal stimulation was used with the following parameters: maximum stimulus intensity 62V, ramp up 1s, on time 1s, ramp down 1s, off time 12s. The stimulus intensity used each day was set to the highest comfortable level for each subject.

The voltage from the stimulator was applied across the skin using 2 round PALS platinum neurostimulation

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electrodes, with a diameter of 50mm. The electrodes were placed on the subject's right leg, over the motor points of the calf muscle. In order to ensure uniform electrode-skin contact the hair under the electrode sites was trimmed prior to electrode placement.

The voltage and current measurements were performed using a battery powered Tektronix TDS 3014B oscilloscope. Two Tektronix P3010 passive voltage probes, in a differential setting, were used for the voltage measurements and a Tektronix TCP202 DC coupled current probe was used for the current measurements, the current probe was coupled with the active electrode lead.

The NI LabVIEW 8.5 software was used to filter and isolate the voltage and current waveforms and obtain their peak values, the Microcal OriginPro 8 software was used for fitting the exponential curves of the measured currents and Mathcad 14.0 was used for complex calculation purposes.

A. Measured Voltage and Current

Each measurement consisted of a measured voltage pulse, the signal applied by the NMES device, and a measured current exponential decay, the human skin response to the stimulation, Fig.1. Each voltage pulse represents the positive going pulse out of a biphasic pulse measured in the 1s on time of the trapezoidal stimulation waveform.

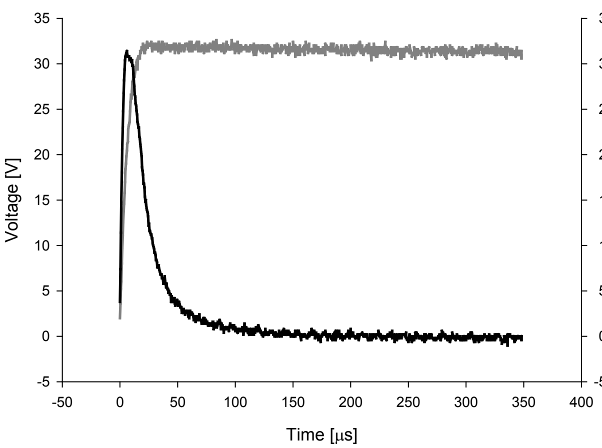

Fig. 1. Measured voltage pulse and current exponential decay for subject 1, day 1, 0 minutes into the stimulation session.

B. Obtaining Voltage Values and the Current Equations

From each measured voltage pulse, a peak voltage value was extracted. Each measured current exponential decay was fitted with a one and two time constants exponential equation, Equations 1 and 2.

$$y = y_0 + A_1 \cdot e^{-\frac{x}{t_1}} \quad (1)$$

$$y = y_0 + A_1 \cdot e^{-\frac{x}{t_1}} + A_2 \cdot e^{-\frac{x}{t_2}} \quad (2)$$

The quality of the fit obtained was given by the χ^2 and R^2 values. We chose to associate the one time constant equation to the human skin equivalent electrical model in Fig. 2A, and the two time constants equation to the human skin equivalent model in Fig. 2B.

C. Obtaining Values for the Human Skin Equivalent Electrical Model Components

The current equations for the one and two time-constant equivalent electrical models were derived.

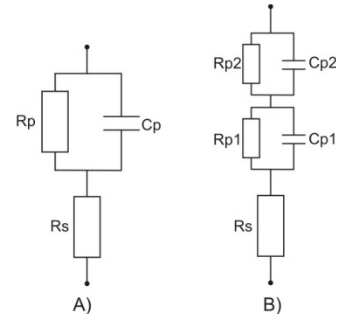

Fig. 2. Human skin equivalent electrical models: A) one time-constant skin equivalent electrical model; B) two time-constant skin equivalent electrical model. R_S is the viable skin resistance, R_P, C_P and R_{P1}, C_{P1}, R_{P2}, C_{P2} are the stratum corneum resistance and capacitance.

Equation 3 is the current equation for the one time-constant equivalent electrical model, Fig. 2A.

$$I(t) = \frac{V}{R_S+R_P} + \frac{V}{R_S+R_P} \cdot \frac{R_P}{R_S} \cdot e^{-\frac{t}{\tau}} \quad (3)$$

where the time constant is:

$$\tau = \frac{R_S \cdot R_P \cdot C_P}{R_S+R_P} \quad (4)$$

Equation 5 is the current equation for the two time-constant equivalent electrical model, Fig. 2B.

$$I(t) = \frac{V}{R_S+R_{P1}+R_{P2}} + \frac{V\cdot(B+C)}{2(R_S+R_{P1}+R_{P2})} e^{-\frac{t}{\tau_1'}} + \frac{V\cdot(B-C)}{2(R_S+R_{P1}+R_{P2})} e^{-\frac{t}{\tau_2'}} \quad (5)$$

where the time constants are:

$$\tau_1' = \frac{2\cdot R_S \cdot R_{P1} \cdot C_{P1} \cdot R_{P2} \cdot C_{P2}}{(R_S+R_{P2})\cdot R_{P1}\cdot C_{P1} + (R_S+R_{P1})\cdot R_{P2}\cdot C_{P2} - \sqrt{A}} \quad (6)$$

$$\tau_2' = \frac{2\cdot R_S \cdot R_{P1} \cdot C_{P1} \cdot R_{P2} \cdot C_{P2}}{(R_S+R_{P2})\cdot R_{P1}\cdot C_{P1} + (R_S+R_{P1})\cdot R_{P2}\cdot C_{P2} + \sqrt{A}} \quad (7)$$

A, B and C are complex functions of R_S, R_{P1}, C_{P1}, R_{P2}, C_{P2}.

Equations 1 and 3 are equivalent and give rise to a system of three equations with three unknowns: R_S, R_P, C_P. By solving this system of equations the values for the one time-constant equivalent electrical model components were obtained.

Equations 2 and 5 are equivalent and give rise to a system of five equations with five unknowns: R_S, R_{P1}, C_{P1}, R_{P2}, C_{P2}. By solving this system of equations the values for the two time-constant equivalent electrical model components were obtained.

III. Results and Discussions

A. Current Curve Fitting Quality

When analyzing the current data for day 1 of the study, for all of the subjects, we noticed that choosing the two time constants exponential equation significantly improved (5.89 times) the quality of the curve fitting in only one case, subject 1, whereas the improvement in the fit quality for the rest of the subjects was insignificant (on average 1.07 times), Fig 3. The only difference between the data for subject 1 and the rest of the subjects was that the amplitude of the applied voltage was >30V for subject 1 and <30V for the rest of the subjects, Table I.

TABLE I
CURVE FITTING QUALITY FOR DAY 1, ALL SUBJECTS

Subject	1(m)	2(m)	3(f)	4(m)	5(f)	6(f)	7(f)	8(m)	9(m)	10(m)	11(m)	12(f)	13(f)	14(f)
Voltage [V]	31.80	23.75	21.41	20.24	24.27	18.35	18.96	21.63	27.33	20.64	18.90	18.52	19.18	24.62
1τ-χ^2	1.359	0.412	0.544	0.392	0.570	0.346	0.312	0.388	0.696	0.666	0.530	0.384	0.440	0.374
2τ-χ^2	0.230	0.338	0.423	0.374	0.381	0.399	0.362	0.231	0.493	0.795	0.557	0.453	0.488	0.320
1τ-R^2	0.988	0.992	0.983	0.976	0.988	0.983	0.990	0.990	0.982	0.994	0.989	0.992	0.994	0.995
2τ-R^2	0.998	0.994	0.987	0.977	0.992	0.981	0.989	0.994	0.988	0.993	0.988	0.991	0.993	0.995

Subjects 1 to 14 (m-male, f-female), voltage values for each, χ^2 and R^2 values for the 1 and 2 time constant (τ) exponential equation fit.

TABLE II
CURVE FITTING QUALITY FOR ALL >30V DATA

Subject (day)	1(1)	1(2)	1(3)	1(4)	1(5)	1(6)	1(7)	3(6)	3(7)	10(4)
Voltage [V]	31.799	31.807	31.815	31.926	31.766	34.302	36.941	32.241	34.839	29.844*
1τ-χ^2	1.359	1.638	1.387	1.300	1.179	1.997	2.694	0.773	0.712	1.541
2τ-χ^2	0.230	0.884	0.313	0.292	0.238	0.344	0.407	0.317	0.264	0.361
1τ-R^2	0.988	0.989	0.995	0.995	0.995	0.992	0.992	0.995	0.996	0.996
2τ-R^2	0.998	0.994	0.999	0.999	0.999	0.999	0.999	0.998	0.999	0.999

Subjects 1, 3 and 10, day in brackets, voltage values for each, χ^2 and R^2 values for the 1 and 2 time constant (τ) exponential equation fit.

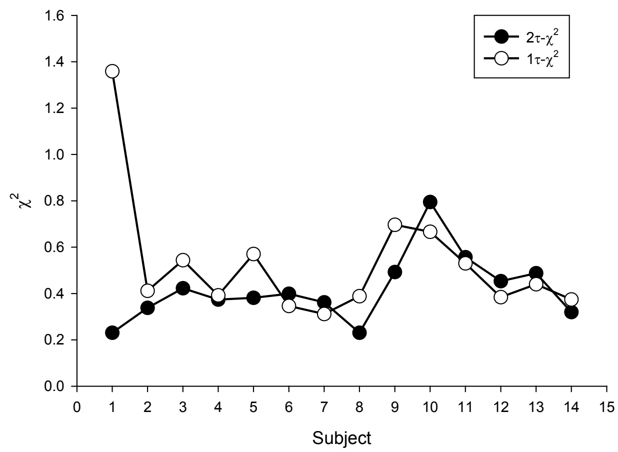

Fig. 3. Quality of fit for day 1 measurements for all subjects. χ^2 values for the one and two time constant exponential equation fit.

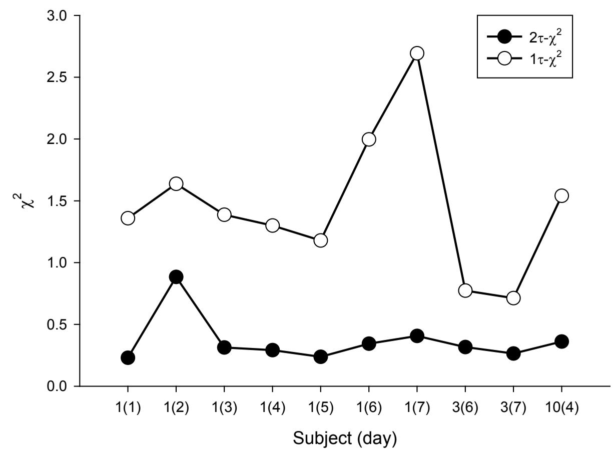

Fig. 4. Quality of fit for over 30V stimulation intensity measurements. χ^2 values for the one and two time constant exponential equation fit.

Previous investigators [6] have found that in the case of applied voltage amplitudes of <30V current flow will mainly be directed through the skin appendages, whereas in the case of applied voltage amplitudes of >30V new pathways are formed in the skin's stratum corneum, through electroporation, thus the current flow has more pathways available and the skin resistance drops drastically.

Based on these previous findings, we supposed that if the applied voltage amplitude was >30V a different conduction mechanism governed the passage of current through the skin, and postulated that this new conduction mechanism adds an extra time constant (resistive-capacitive component) to the skin equivalent electrical model.

We analyzed all the data available that met the criteria that the applied voltage amplitude was >30V. Only one of the 14 subjects found this stimulation intensity comfortable for all of the 7 consecutive days (Subject 1, days 1 to 7), another subject found this stimulation intensity comfortable for 2 days (Subject 3, days 6 and 7), and another for one day (Subject 10, day 4*), Table II.

In agreement with our hypothesis, all of the selected data followed the same rule, the two time constants exponential equation was more appropriate for use (on average 3.99 times) then the one time constant one, Fig 4.

B. Human Skin Equivalent Electrical Model Component Values

The values of the equivalent electrical model components were extracted for all of the >30V data using both the one time-constant equivalent model, Table III, and the two time-constant equivalent model, Table IV.

It is obvious that by choosing the two time-constant model we can isolate the electrical properties of the newly formed pores in the stratum corneum (R_{P1} and C_{P1}) from the initial conduction mechanism that is present both in the <30V and >30V skin electrical response (R_{P2} and C_{P2}). The newly formed conduction pathways offer a much smaller resistance to the passage of current (on average 170Ω compared to 10-20kΩ), and have a significantly larger capacitance (on average 200nF compared to 50-60nF), Table III and IV.

TABLE III

ONE TIME-CONSTANT EQUIVALENT ELECTRICAL MODEL COMPONENT VALUES FOR ALL >30V DATA

Subject (day)	1(1)	1(2)	1(3)	1(4)	1(5)	1(6)	1(7)	3(6)	3(7)	10(4)
Voltage [V]	31.799	31.807	31.815	31.926	31.766	34.302	36.941	32.241	34.839	29.844*
Current [A]	104.067	106.377	104.838	104.920	102.891	106.823	108.665	69.663	77.241	103.086
Rs [Ω]	305.550	299.081	303.846	304.364	308.856	321.567	340.315	462.884	451.246	289.445
τ [μs]	18.695	16.045	17.576	16.827	17.560	17.947	15.928	27.630	27.046	25.123
R_P [kΩ]	14.020	16.231	13.757	9.511	11.375	8.793	13.935	15.342	15.192	8.010
C_P [nF]	62.564	54.692	59.282	57.131	58.500	58.305	48.051	61.509	61.750	89.990

Subjects 1, 3 and 10, day in brackets, voltage and current values for each, τ - time constant values, R_S, R_P, C_P values.

TABLE IV

TWO TIME-CONSTANT EQUIVALENT ELECTRICAL MODEL COMPONENT VALUES FOR ALL >30V DATA

Subject (day)	1(1)	1(2)	1(3)	1(4)	1(5)	1(6)	1(7)	3(6)	3(7)	10(4)
Voltage [V]	31.799	31.807	31.815	31.926	31.766	34.302	36.941	32.241	34.839	29.844*
Current [A]	104.067	106.377	104.838	104.920	102.891	106.823	108.665	69.663	77.241	103.086
Rs[Ω]	305.550	299.081	303.846	304.364	308.856	321.567	340.315	462.884	451.246	289.445
τ'_1 [μs]	49.172	38.036	44.660	48.327	46.512	41.219	37.199	64.423	60.444	58.391
$R_{P!}$ [Ω]	181.360	120.033	153.479	168.098	163.743	195.232	197.076	212.803	183.168	131.831
$C_{P!}$ [nF]	186.405	235.637	203.901	208.965	203.122	134.003	128.066	198.798	222.192	299.476
τ'_2 [μs]	11.833	11.132	11.758	11.989	11.832	10.033	9.168	18.158	18.129	16.664
R_{P2} [kΩ]	27.786	25.015	22.843	13.864	17.869	14.555	25.389	29.478	25.814	12.663
C_{P2} [nF]	57.532	51.048	56.822	56.366	55.601	51.714	41.481	61.066	61.618	88.322

Subjects 1, 3 and 10, day in brackets, voltage and current values for each, τ'_1, τ'_2 - time constant values, R_S, R_{P1}, C_{P1}, R_{P2}, C_{P2} values.

IV. CONCLUSIONS

Our findings show that electroporation can be detected from pulsed voltage measurements when the current exponential response of the human skin is best modeled by a two time constants exponential equation. This second time constant is representative of a second resistive-capacitive component in the human skin equivalent electrical model that could be associated to the electrical properties of the newly formed pores in the stratum corneum.

This technique could be of use in detecting electroporation for practical applications such as electrically controlled transdermal drug delivery.

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In the case of Subject 10 day 4 five of the seven measured voltages were >30V and two of the measured voltages were just bellow 30V (5 minutes into the stimulation session V=28.551V and 15 minutes into the stimulation session V=27.559V).