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Electrical Impedance Spectroscopy Study of Biological Tissues

D.A. Dean^{1,*}, T. Ramanathan², D. Machado¹, and R. Sundararajan^{1,3,**}

¹*Division of Pulmonary & Critical Care, Northwestern University Medical School, Chicago, IL-60611, e-mail: dean@northwestern.edu*

²*Mechanical Engineering Dept., Northwestern University, Evanston, IL-60208, e-mail: trammathan@northwestern.edu*

³*Electronics & Computer Engineering Technology, Arizona State University, Mesa, AZ-85212*

Abstract

The objective of this study was to investigate the electrical impedance properties of rat lung and other tissues ex vivo using Electrical Impedance Spectroscopy. Rat lungs (both electroporated and naïve (untreated)), and mesenteric vessels (naïve) were harvested from male Sprague-Dawley rats; their electrical impedance were measured using a Solartron 1290 impedance analyzer. Mouse lung and heart samples (naïve) were also studied. The resistance (Real Z, ohm) and the reactance (Im Z, negative ohm) magnitudes and hence the Cole-Cole (Real Z versus Im Z) plots are different for the electroporated lung and the naïve lung. The results confirm the close relationship between the structure and the functional characteristic. These also vary for the different biological tissues studied. The impedance values were higher at low frequencies compared to those at high frequencies. This study is of practical interest for biological applications of electrical pulses, such as electroporation, whose efficacy depends on cell type and its electrical impedance characteristics.

Keywords

impedance; spectroscopy; tissue; rat; mice; lung; mesenteric vessel; heart

Introduction

Tissue electrical impedance is a function of its structure and it can be used to differentiate normal and cancerous tissues in a variety of organs, including breast, cervix, skin, bladder and prostate [1]. Electrical Impedance Spectroscopy (EIS) has been widely used to assess the condition of animal tissues, both in vivo, in vitro and ex vivo and for various other applications [2-13]. This method is useful to characterize cellular changes quantitatively [5,11,13]. The electrical impedance of a volume of tissue at a series of frequencies provides information about the cell population. Predominantly, the characteristics and integrity of the population's plasma membranes, cell volumes, and intra and extracellular conductivities influence the impedance spectrum. Thus, EIS can be used as a method of identifying and following detectable cellular responses, in ex vivo, in vivo and in vitro [5,9] to take advantage of this prognostic information. In general EIS provides impedance information with respect to a wide range of frequency,

*Currently at University of Rochester

**Currently at ECET, Purdue University, 401 N. Grant St. W. Lafayette, IN 47907, e-mail: raji@purdue.edu

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which is not available via other non-invasive diagnostic techniques and which can be used for treatment purposes [2,12]. For example, impedance spectroscopy was used to differentiate normal and malignant prostate tissue by Halter et al [1]. It was used to study the malignant and normal breast tissues of 26 patients by Kerner et al. [2]. It was used to determine the state of organs by Gersing et al [12]. The impedance data was used to characterize tissues and their changes during ischemia. Electrical impedance spectroscopy was also used to monitor the yeast cell growth [11]. Several researchers are interested in using impedance spectroscopy to better understand the electric and dielectric properties of tissues as they provide crucial information needed to understand the effects of electrical pulses, such as electrical stimulation and electroporation [15,3].

Cell membranes seem to interact with applied pulsed electric fields causing intramolecular transitions and intermolecular processes that lead to structural reorganization of the cell membranes [10]. Applying high voltage pulses (Electroporation or Electroporabilization, EP) in cultures or in vivo causes dielectric breakdown of the cell membranes. These phenomena can be studied and quantified using EIS. The work reported here explores the use of EIS in studying quantitatively the electrical properties of various naïve tissues and an electroporated tissue, because the electrical impedance of a volume of tissue at a series of frequencies provides information about the cell population and electroporabilization affects the membrane resistivity, and is a direct consequence of the dielectric breakdown of the membrane barrier. In the present investigation, the electrical properties, such as the resistance (Real Z) and the capacitive reactance (Im Z) of the rat and mouse tissues were investigated, as the efficacy of the electroporation depends on the type of cell or tissue and its electrical properties.

Electrical Properties of Biological Tissues

The body can be considered as a composite volume conductor comprising a number of spatially distributed tissues with differing electrical properties [14]. Unlike metallic conductors, electrical conduction within biological tissues is due to ions. In the presence of an electric field, a conduction current I_c will develop due to the movement of mobile ions within the aqueous biological medium [15]. This current is therefore related to the ionic content and ionic mobility of the particular tissue. It is expressed in terms of the tissue's conductivity σ . The ion mobility is also temperature dependent.

Bound charges within tissues give rise to complex dielectric properties, and thus displacement currents I_d will contribute to the time-varying electrical behavior. These bound charges include electrical double layers at membrane surfaces and polar molecules, such as proteins. Both conductivity and relative permittivity vary widely between different biological tissues and these parameters also vary with the frequency of the applied field. The permittivity is related to the extent to which the bound charges can be displaced or polarized under the influence of the electric field. Each polarizable entity within the tissue will exhibit its own characteristic response and thus a distribution of relative permittivities will give rise to a complex function of frequency of the form [16]:

$$\epsilon^*(\omega) = \epsilon_\infty + (\epsilon_s - \epsilon_\infty) / (1 + j\omega\tau) \quad (1)$$

where ϵ_∞ is the high frequency permittivity at which the polarizable entities are unable to respond, ϵ_s is the low frequency permittivity where polarization is maximal, ω is the angular frequency, and τ is the characteristic relaxation time of the tissue under study. A dielectric dispersion is therefore associated with biological tissues [17] in which the relative permittivity decreases with increasing frequency. However, the displacement current is proportional to the applied field frequency and so these two opposing factors lead to a complicated frequency behavior. In general, three discrete regions of dispersion can be identified in biological tissues as depicted by Schwan [17]. These are commonly defined as:

- Alpha dispersion (10Hz to a few kHz), associated with tissue interfaces, such as membranes
- Beta dispersion (1kHz to several MHz), associated with the polarization of cellular membranes and protein and other organic macromolecules
- Gamma dispersion (>10GHz), associated with the polarization of water molecules.

Fig. 1 shows an illustration of these frequency dispersions [17].

Materials and Methods

Rat Lung Tissue Samples

Male Sprague Dawley rats (350g) were anesthetized and euthanized using a mixture of isoflurane and oxygen. The naïve lungs were removed and preserved in phosphate buffer saline solution (PBS) (at 10% concentration) and were carried to the impedance spectroscopy facility immediately. Fig. 2a shows a naïve rat lung sample.

In the case of electroporated lung, the rat was anesthetized and was kept under a ventilator. A series of eight square wave pulses of 100 μ s duration each (at 1s interval) were administered to the animals using Medtronic pediatric electrodes and an ECM830 Electroporator (BTX, Harvard Apparatus). An electric field strength of 100V/cm was applied. The voltage applied, the number of pulses and pulse duration were similar to those used early in previous studies as these were found to give an optimal efficacy of gene transfection [18]. The electrodes were placed externally on either side of the chest, which had been wetted with 70% ethanol. Later, the rat was anesthetized and euthanized and the lungs were harvested for Impedance Spectroscopy analysis. Fig. 2b shows an electroporated rat lung with DNA distribution. The electroporated lung was reddish in color compared to the naïve lung which was pinkish.

Rat Mesenteric Vessels

The mesentery includes a thin tissue sheet connecting the intestines to the main vascular tree and encompasses numerous vascular bundles (Fig. 3) [19]. Each bundle usually has multiple minor blood vessels with one larger artery and one larger vein. Surrounding each vessel is fatty tissue and extracellular matrix that prevents twisting and kinking of the vessels as the intestines move. Electroporation study was performed on rat mesenteric vessels by Young et al., as in the laboratory research setting, the vasculature provides an excellent medium for *in vivo* studies [20]. It exhibits many valuable characteristics as a model for gene delivery. Three main cell types (endothelial cells, smooth muscle cells, and fibroblasts) comprise the arteries and veins. The large surface area of the mesenteric vasculature is easily accessible for site specific treatment, and the vascular tree contains many neurovascular bundles that can be identified by anatomical separations.

Following a procedure similar to that for naïve rat lungs, individual rat mesenteric vessels were cut and removed and measurements were made.

Mouse Lung and Heart Tissue Samples

Female Balb/c mice (15-18g) were anesthetized and euthanized by pentobarbital overdose and cervical dislocation [18]. The naïve lung, and heart were removed and preserved in PBS solution (10%) and the impedance spectroscopy measurements were performed.

All experiments were conducted in accordance with institutional guidelines in compliance with the recommendations of the Guide for Care and Use of Laboratory Animals (at Northwestern University).

Electrical Impedance Spectroscopy

AC impedance at room temperature was measured using a Solatron 1290 (Hampshire, UK) impedance analyzer with 1296 dielectric interface. The samples were sandwiched between the two electrodes of a Solatron 12962 sample holder. Animal tissues were scanned at either 81 or 41 frequency points over the frequency range 0.01Hz to 1MHz. Electrical impedance was displayed as Real Z (R, resistive component in Ω) and Im Z (capacitive reactance component in negative Ω).

Results and Discussion

Rat lung Tissues

Bode Plots (Frequency versus Z, R, and X)—Biological tissue structures exhibit two electrically conducting compartments, the extra- and intracellular spaces separated by insulating membranes. The conduction of electric current through such a structure is highly frequency dependent [12]. The conductivity (inverse of resistivity) reflects the conduction properties of the tissue. Therefore, the tissue impedance spectrum in the frequency ranges up to about 1MHz reflects the properties of the structures. This frequency-dependent relationship between impedance (z), conductivity (σ) and relative permittivity (ϵ_r) is given by the expression [7]:

$$Z = Z' + j\omega Z'' = 1 / (\sigma + j\omega \epsilon_0 \epsilon_r) \quad (2)$$

where Z is the total (complex) impedance, Z' and Z'' are the real and imaginary components of Z respectively, ω is the radial frequency, and ϵ_0 is the permittivity of free space. Both Z' and Z'' were measured, from which the conductivity and relative permittivity can be calculated. It was assumed that the tissues have no or negligible inductive influence. Electroporation affects the membrane resistivity and it is a direct consequence of the dielectric breakdown of the membrane barrier. We investigated the impedance method as an approach to understanding tissue structures and their characteristics under naïve and electroporation conditions.

The total impedance variation Z of rat lung (naïve) from 10Hz to 10kHz is shown in Fig. 4. There is a steep increase in impedance at low frequencies, a common feature in organs whose cells are interconnected [12]. This was contributed by both the real and imaginary components as illustrated in Fig. 5. This is because an intact cell membrane is similar to an ultrathin capacitor of high resistance that envelops the intracellular fluids. At low frequencies of applied electric field, the membrane is highly resistant and a low electric current will travel in the extracellular fluid surrounding the cells, hence the impedance is very high. As the frequency increases, this impedance decreases as the resistance drops due to its predominant capacitive behavior. At very high frequencies, since $Z'' = X_c = 1/2\pi fC$ and Z' is very small, the membrane impedance approaches zero and the membranes appear as a short circuit; the electric field lines pass more uniformly through the tissue structure as the impedance decreases towards its minimum value [4,11]. The naïve rat lung studied exhibited this behavior as seen in Figs. 4 and 5. It also perfectly fits the power law frequency dependency reported in the pioneering work of Schwan [20]. A power-law constant of -0.3 to -0.5 is reported in that work as the frequency increases from below 1 to more than 10kHz. In our study, we obtained a power law constant of -0.44 ($R^2=0.9974$) (Fig. 6a) illustrating a good correlation with the above reported values by Schwan [21]. It is also reported that in general, biological tissues exhibit two distinctly different dispersions, α dispersion at low frequencies and β dispersion at high frequencies [15,12,22, 23]. We also obtained similar data for the naïve rat lung studied: α dispersion around 100Hz and β dispersion around 10kHz (Fig. 6).

Figs. 6a, b, and c show a comparison of the impedances of the electroporated and the naïve lung tissues at various frequency ranges, 10-10,000Hz (Fig. 6a), 1-1000Hz (Fig. 6b), and 1kHz-1MHz (Fig. 6c). The EP impedance values are generally lower than the naïve impedance values [23]. This reduction in impedance magnitudes for the electroporated tissue is due to the increase in permeability of the membranes, since the application of electrical pulses causes transient dielectric breakdown (reversible electroporation), and hence increase in the conductivity. While the magnitudes vary, the trend of the impedances of the EP and naïve tissues are similar. The electroporated tissues also follows the inverse power-law with power-law constants of 0.52 ($R^2=0.9993$), 0.56 ($R^2=0.9989$), and 0.62 ($R^2=0.9164$) for the three frequency ranges respectively compared to 0.43 ($R^2=0.9978$), 0.44 ($R^2=0.9974$), and 0.45 ($R^2=0.9993$) for the naïve tissues. The EP tissue also exhibits α and β dispersions at all frequency ranges similar to that of naïve. These results correlate well with those obtained for human stratum corneum in vitro for a frequency range between 10Hz and 1MHz [3]. The impedance magnitudes were higher for the human sample. Fig. 7 shows the individual real and imaginary components of impedance of the electroporated lung sample over the frequency range 10Hz to 1MHz.

Cole-Cole Impedance Plots—Figs. 8a and b show the Real Z versus Im Z complex plane locus, called as Cole-Cole impedance plots [3, 5, 7, 13, 21, 22] for the naïve and the electroporated lungs. Here, the imaginary part of the complex impedance of the material under study is plotted against the real part, each point being characteristic of one frequency of measurement. The low frequency data are on the right side of the plot (having higher magnitudes) and the high frequency data are on the left as the impedance falls with increase in frequency. For a truly complex impedance, the Cole-Cole impedance plot will be a semi-circle, due to second order variation ($r^2 = a^2 + b^2$, equation of the circle). In the present case, it approaches a semi-circle. The electroporated lung impedance magnitudes are smaller than the naïve lung impedance values due to the increase in permeability. These results correlate well with the results reported for human skin [3].

Tan δ variation with frequency—Dielectric physics [24] indicates that the loss tangent in the low frequency range is caused by electrical conduction current, and, can be expressed as

$$\tan \delta = \frac{\sigma}{2\pi f \epsilon_0 \epsilon_r} = \frac{I_c}{I_d}, \quad (3)$$

where f is the applied frequency, σ is the electrical conductivity, ϵ_0 is the dielectric permittivity of free space, ϵ_r is the relative permittivity. Thus, it is an expression of the frequency dependence of the relative dielectric constant, ϵ_r or the capacitance C and the conductivity σ . Due to water in the tissues, more H^+ , $(OH)^-$, and other ions contribute to the conductance. This leads to a higher value of σ . Additionally, since water has a high relative permittivity, the presence of water will increase ϵ_r . Both σ and ϵ_r vary with frequency since the conductivity and dielectric constant are frequency dependent in a dielectric. Conduction current will contribute at high frequency as well, although polarization loss also becomes significant at high frequency. Thus there is a complex variation of $Re Z$ and $Im Z$ with frequency and hence in $\tan \delta$ also. Fig. 9 shows the variation of $\tan \delta$ with frequency for both naïve and EP rat lung tissues. In this study, $\tan \delta$ was calculated as the ratio of real and imaginary parts of the impedance at any particular frequency. A large $\tan \delta$ indicates that there is more dielectric absorption.

Biological samples, such as cell suspensions and tissues are highly conductive. Therefore their $\tan \delta$ values increase as the frequency decreases as the resistive component of their impedance dominates [21]. Such a behavior was seen in this study for naïve and electroporated rat lungs. Tables 1 and 2 also show variations of $Re Z$, $Im Z$, and total Z magnitudes for various frequency

ratios for these tissues. The electroporated tissue has lower ratios than the naïve tissue. While there is a decrease in the ratio of the $\tan \delta$ ratio value with increasing frequency for naïve rat lung, EP lung shows, first a small increase and then a decrease with increase in frequency. Overall, net response is a complex behavior. Physically, the EP lung tissue was more reddish compared to the naïve one, which was more pinkish. The statistical significance of the above results ($p < 0.05$) is given in Table 3.

Rat Mesenteric Vessels—Figs. 10 and 11 illustrate the impedance dispersions of naïve rat mesenteric vessel 1. The total Z curve obeys the power law (with a constant of -0.52 with $R^2 = 0.9994$). There is α dispersion around 10Hz and β dispersion around 5kHz. Fig. 12 shows the corresponding Cole-Cole impedance plot for this tissue. Five vessels were tested and Fig. 13 shows the Real Z for these. There is some difference in the magnitudes between the five vessels (from the same animal). This is typical for biological tissues, where each sample exhibits slightly different magnitudes. Different contents of water, fat, connective tissue may cause variations. Moreover, local tissue anisotropy and inhomogeneities would also affect tissue impedance. Table 4 gives a the variation of Re Z , Im Z , total Z , and $\tan \delta$ along with frequency variation for mesenteric vessel 1.

Mouse Lung and Heart—Similar analyses were also performed for mouse lung and heart tissues and sample results are illustrated in Figs. 14 and 15. Mouse lung and heart also follow the power law [20]. Fig. 14a and b show their Re Z and Im Z spectra with alpha and beta dispersions and Fig. 15a and b show the Cole-Cole impedance plots exhibiting second order behavior as other tissues. Tables 5 and 6 give the ratios of the various parameters at different frequency ratios. Fig. 16 shows a comparison of the various samples, rat lung, vessel, and mouse lung and heart. The impedance values are the highest for the rat lung and the lowest for the mouse lung. Table 7 illustrates the statistical significance of these data ($p < 0.05$). The difference between mouse heart and lung seems to be not statistically significant ($p = 0.14$).

Equivalent Circuit—Electrical Impedance spectroscopy data is also analyzed by fitting it to an equivalent circuit model, consisting of resistances and capacitances in series and parallel combinations such that its impedance matches the measured data [3,12,25]. Sample R and C values computed for rat and mouse lungs are shown in Table 8 over the frequency range 10-10,000Hz. R and C values were obtained for other frequencies as well as for other tissues (data not shown). Simple combinations of series and parallel R and C elements that give almost an half circle Cole-Cole plot when the imaginary part versus real part was plotted for human skin [3]. Fig. 17a shows the model used where, C_a is the capacitor modeling the intercellular bilayer membranes, R_a is the modeling of the electrolyte between membrane structures and R_p represents the resistance of the dc-path across the stratum corneum. There are also other electrical models of biological cells or tissues. Since biological cells are extremely complex, a number of combinations of series and parallel resistors and capacitances may be needed. There is no unique equivalent circuit that could describe any cell completely. Fig. 17b shows an electrical model of a biological cell that simulated closely the cell electrical characteristics at low and high frequencies [25]. Here, the plasma membrane of the cell was modeled as resistors R_{c1} and R_{c3} , in combination with capacitors C_{m1} and C_{m2} to represent the leaky dielectric nature. The nucleus was modeled using resistor R_n along with capacitors C_{n1} and C_{n2} . These are connected in parallel with R_{c2} , the conductive cytoplasm. The respective values used are indicated. Similar RC models were also used by other researchers [12,13]. Fig. 17c shows the model reported by Gersing [12] for three interconnected cells of porcine liver used in their study on determining the status of the organs using impedance spectroscopy. Here, the extracellular pathway is represented by resistor R_e , the membrane by C_m , the cytosol by R_{i1} , the gap junction by R_{g1} into the next cell. Since, the scope of our work is to characterize various tissues using their impedance values over a range of frequency, modeling will be done in future.

Conclusions

The electrical properties of tissues in various frequency ranges are determined by the cellular components and the dimensions, internal structure and arrangements of the constituent cells. Hence, tissues with different cellular structures will give rise to characteristic impedance spectra. The knowledge of electrical properties of biological tissues has been one of the keys to increasing our understanding of their structure and function. Electrical impedance of biological tissues is a complex quantity combining resistance and capacitance and it depends on the frequency of the ac voltage applied. This is because they have components that have both conductive and charge storage properties. By studying the electrical impedance of various tissues over a frequency range using EIS, its frequency-dependent electric and dielectric behavior can be determined and used for various applications including pathology, prognosis, diagnosis and healing using electrical pulses. The use of impedance spectroscopy electrical characterization is a novel approach to comprehend underlying operative phenomena in a number of material systems, including biological tissues. In this research, for the first time, the electrical properties of rat lungs, both naïve and electroporated were studied using EIS for a range of frequency. In addition, naïve rat mesenteric vessels, mouse lung and heart were measured. The variation of impedance with frequency follows the negative, fractal, power law, reported in the pioneering work of Schwan [21]. Typical frequency spectra for these various biological tissues showed the real part of the impedance which is associated with resistive pathways across the tissues and the imaginary part of the tissue which is associated with capacitive pathways, such as membrane structures. The real part was large at low frequencies, such as 10Hz or lower. With increasing frequency, the real part of the impedance decreased and the imaginary part became more dominant. At high frequencies, the imaginary part of the impedance becomes small as frequency is high in the expression for capacitive reactance, given as $X_c = 1/j2\pi fC$, where C is the capacitance (of the membranes), and hence the net impedance is almost zero. At very high frequencies, due to the very small time constants, current doesn't flow, but only moves back and forth between membrane surfaces and hence neither the resistive pathways nor the capacitive pathways of the membranes have time to play a role [3]. The current is limited by the small resistance of the membranes. All the results obtained correlate very well with the results reported previously by Schwan [21], and Pliquett and Prausnitz [3]. These results are valuable to understand better the variation in the electroporation parameters (magnitude and duration) in different tissues and the need for optimizing these for each tissue as the electrical properties of biological tissues are related to their physiological, morphological, and pathological conditions and the electric field requirement varies from cell to cell [26,27].

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Fig. 1. An idealized plot of the frequency variation of the relative permittivity of a typical biological tissue [17].

Fig. 2a

Fig. 2b

Fig. 2.

Fig. 2a Naïve Rat Lung (pinkish in color).

Fig. 2b Electroporated rat lung with DNA distribution (reddish in color).

Fig. 3.

The rat mesenteric vascular tree. Numerous neurovascular bundles extend radially outward from the mesenteric vascular trunk to the intestine. A thin membrane (mesentery) spans the space between the bundles [19].

Fig. 4. Variation of Impedance Z with frequency (10Hz to 10kHz)-Rat Lung-Power law Constant is -0.44 ($R^2 = 0.9974$).

Fig. 5. Variation of Real Z and Imaginary Z with frequency for Naïve Rat lung from 10Hz – 1MHz with α and β dispersions.

Fig. 6a

Fig. 6b

Fig. 6c

Fig. 6.

Fig. 6a Impedance Variation of Electroporated versus Naïve Rat Lung Tissues from 10 to 10,000 Hz.

Fig. 6b Impedance Variation of Electroporated versus Naïve Rat Lung Tissues from 1 to 1,000 Hz.

Fig. 6c Impedance Variation of Electroporated versus Naïve Rat Lung Tissues from 1kHz to 1MHz.

Fig. 7. Variation of Real Z and Im Z with frequency for Electroporated rat lung from 10Hz – 1MHz with α and β dispersions.

Fig. 8.
Cole-Cole Impedance Plot (Re Z vs Negative Im Z) for Naïve and EP Rat Lungs.

Fig. 9.
Variation of Tan δ with Frequency for EP (top curve) and Naive (bottom curve) Rat lungs.

Fig. 10. Variation of Impedance Z with frequency (10Hz to 10kHz) – Rat Vessel-Power law constant is -0.52 ($R^2 = 0.9994$).

Fig. 11. Variation of Real Z and Imaginary Z with frequency from 10Hz – 1MHz for Naïve Rat Mesenteric Vessel.

Fig. 12. Cole-Cole Impedance Plot (Re Z vs Negative Im Z) for Rat Vessel for 0.01Hz – 1MHz frequency range showing second order variation.

Fig. 13. Variation of Resistance for Five (from the same animal) Naïve Rat Vessels with frequency with alpha (about 100Hz), and beta (about 10kHz) dispersions.

Fig. 14.
Variation of Real Z and Im Z with frequency from 10Hz – 1MHz for Naïve Mouse

Fig. 15.
Cole-Cole Impedance Plot (Re Z vs Negative Im Z) for Mouse Lung for 0.01Hz – 1MHz frequency range

Fig. 16.

Variation of Total Impedance Z with frequency – Comparison of Rat Lung (top curve), Rat Mesenteric Vessel (2nd curve from top), Mouse Lung (3rd curve from top), and Mouse Heart (bottom curve). They all obey Power-law.

Fig. 17.
Electrical Models of Biological Tissues
(a) Simple RC series/parallel Model of Human Skin Membrane [3]
(b) Complex RC Circuit of a biological cell - For details, refer [25]
(c) RC Circuit of a Porcine Liver [12]

Table 1

Naïve Rat Lung Impedance Data

Ratio	Total Z	Real Z	Im Z	Tan Delta
10Hz/100Hz	2.64	2.81	2.41	1.16
100Hz/1000Hz	3.08	2.96	3.23	0.91
1000Hz/10000Hz	2.30	2.01	3.13	0.64

Table 2

Electroporated (EP) Rat Lung Impedance Data

Ratio	Total Z	Real Z	Im Z	Tan Delta
10Hz/100Hz	1.84	1.81	1.97	0.92
100Hz/1000Hz	1.95	1.94	1.99	1.00
1000Hz/10000Hz	1.74	1.71	2.00	0.85

Table 3

Statistical Significance of naïve and EP Rat lung measurements

Difference	Degree of Freedom	t	p
Naïve Z - EP Z	40	4.07	0.0002
Tan delta Naïve –Tan delta EP	40	-2.63	0.0121
Naïve Real – EP real	40	3.82	0.0005
Naïve Im – EP Im	40	4.99	<0.0001

Table 4**Rat Mesenteric Vessel Impedance Data Analysis**

Ratio (f1/f2)	Total Z	Real Z	Im Z	Tan Delta
10Hz/100Hz	3.59	3.25	3.84	0.84
100Hz/1000Hz	3.30	3.14	3.45	0.91
1000Hz/10000Hz	3.01	2.54	3.88	0.65

Table 5

Mouse Lung Impedance Data Analysis

Ratio	Total Z	Real Z	Im Z	Tan Delta
10Hz/100Hz	3.44	2.86	3.96	0.72
100Hz/1000Hz	2.87	2.67	3.14	0.85
1000Hz/10000Hz	2.59	2.29	3.36	0.68

Table 6

Mouse Heart Impedance Data Analysis

Ratio	Total Z	Real Z	Im Z	Tan Delta
10Hz/100Hz	2.05	3.55	2.52	0.58
100Hz/1000Hz	1.83	2.38	1.93	0.77
1000Hz/10000Hz	1.63	2.33	1.70	0.70

Table 7

Statistical Significance of Naïve Tissues measurements

Combination	T-value	p-value
Rat Lung vs. Mouse Lung	4.62	0.0003
Rat Lung vs. Rat Vessel	5.92	<0.0001
Rat Lung vs. Mouse Heart	3.86	0.0015
Mouse Lung vs. Rat Vessel	-3.62	0.0025
Mouse Lung vs. Mouse Heart	-1.56	0.1404
Rat Vessel vs. Mouse Heart	2.63	0.019

Table 8

R and C Values of rat and mouse lungs

Frequency, Hz	Rat Lung Electroporated		Rat Lung Naive		Mouse Lung Naive	
	Resistance, R Ω	Capacitance, C μ F	Resistance, R Ω	Capacitance, C μ F	Resistance, R Ω	Capacitance, C μ F
10.0	3565	3.42	6117	3.37	1441.0	11.05
15.8	2778	2.84	5080	2.52	1146.0	8.77
25.1	2183	2.36	4181	1.88	921.1	6.88
39.8	1728	1.96	3401	1.41	747.7	5.35
63.1	1374	1.61	2737	1.06	635.0	3.97
100.0	1096	1.31	2180	0.81	503.7	3.16
158.5	874	1.06	1728	0.63	415.3	2.42
251.2	695	0.86	1370	0.50	342.3	1.85
398.1	552	0.69	1096	0.40	281.0	1.42
631.0	438	0.56	890	0.32	196.0	1.29
1000.0	348	0.45	736	0.26	188.4	0.84
1585.0	279	0.37	622	0.21	155.1	0.65
2512.0	226	0.30	535	0.17	129.1	0.49
3981.0	187	0.25	467	0.14	109.2	0.37
6310.0	158	0.21	412	0.11	94.0	0.27
10000.0	137	0.17	366	0.08	82.3	0.19