Estimation of Cole Parameters in ex-vivo tissues as a function of degradation time

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Abstract. Bioimpedance spectroscopy has been used to evaluate and characterize the integrity of different tissues and organs, as well as to detect tissue structural alterations. The tissue electrical model alterations as an influence of the anisotropy of the tissue structure and its intrinsic metabolism on the degradation dynamic have not been completely understood. In this work, the dynamic of Cole parameters was estimated as a function of degradation time in ex vivo skeletal muscle, skin, and white adipose tissues. The results indicate a non-linear behavior function of the Cole parameters through degradation progress in the three evaluated tissues, and such non-linearities might be associated with different capacities of adaptation to anaerobic metabolism, energy production, and water content under stress conditions.

1. Introduction

Bio-impedance ($Z$) of a biological medium is defined as the relation between a specific voltage and the electric current that it generates ($Z = V/I$). This is a function of frequency and characteristics of the biological medium in which the current flows \cite{1}. Bio-impedance is a complex number, which has the Electrical Resistance ($R$) as its real component and the Electrical Reactance ($X_C$) as its imaginary component, both of them expressed in Ω \cite{2}. Bio-impedance measurements are based on the injection of a low-intensity alternating current in biological mediums and organic tissues. Obtaining these measurements is possible because tissues exhibit conductive and dielectric properties; in other words, they contain free moving electric charges as well as fixed ones \cite{3}. This happens because the cell is a rather complex structure conformed by a membrane constituted by a phospholipidic bilayer in which various proteins distributed within the membrane act as channels for ionic exchange \cite{4}. The components of the cell membrane allow it to act as a dielectric interface, assimilating the two plaques of a condenser. The electrical properties of tissue could be described considering that, at low frequencies, the electric current flows through the extracellular fluids of the tissue while, at high frequencies, the current flows through both extracellular and intracellular fluids.

When the tissue is under the action of an electrical field, charged ions move, producing electrical currents with their displacement. The response of biological tissues to the action of an electric field depends on the characteristics of their structures, composition, dipoles, and capacity of formation and orientation \cite{5}. Conductivity is a rather crucial factor when analyzing the aforementioned response, since it defines the amount of electricity that could flow and it is directly proportional to the concentration of ions dissolved within the biological medium \cite{2}. Relative permittivity ($\varepsilon_r$) on the other
hand, explains why charges in the dielectric move when a field of univarty amplitude is applied; in other words, the capacity of the biological medium to be polarised by an electrical field [3]. Electrical resistance ($R$) is another important factor. This could increase as a consequence of changes in the concentration of ions, viscosity, temperature, or composition of the medium, as well as the separation of the measurement electrodes or changes in the concentration of non-conductive substances. The electrical resistance could also increase if the area of effectiveness in the biological medium decreases [2]. According to [6], there is a disparity in dielectric measurements between living and excised tissues (after 48h) and this occurs because ionic conductivity is dependent on the blood content in the tissue. Another point that influences the aforementioned disparity is the anisotropy that appears in tissues such as skin and muscle, since they have electrical routes preferred by the movement of ions. On the other hand, white adipose tissue lacks these routes but if it has higher water content, therefore its permittivity will be higher [7].

Electromagnetic field changes very slowly at low frequencies for dipoles to reach equilibrium before the inversion of polarity in the field reaches equilibrium. At certain frequency, increased viscosity of the medium also stops the response of dipoles to the electric field because. This produces energy dissipation and irradiation of heat as the response of the medium is delayed. Three dispersions within the spectrum of frequencies can be distinguished in the different biological mediums [2]: a) a first dispersion known as alpha dispersion, frequencies of some hundreds of kHz, where the conductivity of the tissue is subject to the conduction of electrolytes within the extracellular space; a second dispersion (beta), at frequencies higher than the first one, where the relative permittivity reaches extremely high values (tens of millions), becoming more noticeable in conductivity rather than permittivity [8]; and, finally, a third dispersion known as the gamma dispersion, at microwave frequencies (over 1 GHz) due to the rotation rate of the water in tissue. This dispersion becomes centered at 20 GHz and it is the same as the one found in liquid water [8].

Several equivalent electrical models for tissue characterization have been proposed in different RC elements configurations. Extracellular resistance ($R_2$) in series with parallel intracellular resistance ($R_1$) and cell membrane capacitance ($C_1$) is one of the simplest and traditional models (figure 1). In this case, the complex multifrequency tissue impedance responses are in the form of a circular arc with depressed centers of a circle, when tissue reactance is plotted as a function of equivalent series resistance in the complex impedance plane [9]. This graph has frequency crossovers at $R_0$ and $R_\infty$. These arcs are modeled with the help of a constant phase element (CPE), and it is the principle on which Cole's impedance model is based [9]. In this work, we used this electrical model to characterize the studied tissues. The dynamic of Cole parameters were estimated as a function of degradation time in ex-vivo skeletal muscle, skin, and white adipose (fat) tissues.

In this article, we estimated the dynamics of Cole's parameters in function of the degradation time in skeletal muscle ex vivo as well as skin and white adipose tissue. The results indicate a non-linear behavioral function of Cole's parameters through progress in the degradation of the three tissues examined. The previously mentioned non-linearity could be associated with different capacities in regard to adaptation to anaerobic metabolism, energy production, and water content under conditions of stress.
Figure 1. Equivalent electrical model for tissue characterization. Extracellular resistance \((R_2)\) in series with parallel intracellular resistance \((R_1)\) and cell membrane capacitance \((C_1)\).

2. Methodology

2.1. Ex vivo tissue
Two pig \textit{ex vivo} specimens of anus and perianal tissue were obtained from the local slaughterhouse. For the extraction of samples, perianal tissue was divided into four quadrants and in each quadrant, a portion of the muscle, adipose tissue (fat), muscle, and skin was selected to perform electrical bioimpedance measurements. Three electrical bioimpedance measurements were taken; immediately after dissection \((t_1 = 0 \text{ h})\), five hours later \((t_2 = 5 \text{ h})\) and ten hours later \((t_3 = 10 \text{ h})\).

2.2. Bioimpedance measurements
The impedance of the sample was measured with a Mark 3 bioimpedance spectrum, which applies currents of 50 \(\mu\text{A}\). Measurements were taken for each tissue at frequencies 1, 2, 5, 10, 20, 50, 100, and 200 kHz. Figure 2 shows the application on the tissue of the 5.5 mm diameter pencil-shaped probe that was used, which contains 4 gold electrodes of 1 mm diameter in its tip (figure 3). All data obtained was organized according to the type of tissue and frequency, and the mean was calculated using Excel.

Figure 2. Experimental setup. Probe for multifrequency bioimpedance measurements in \textit{ex vivo} tissue.
2.3. Analysis of data.
Experimental values from impedance between 1 and 200 kHz were heuristically adjusted to a mathematical model from a CPE, which allowed us to model the impedance behavior of the electrode-electrolyte interface in series with Cole's impedance equation. This was done with the help of Matlab software (1) [10] [11], using equation 1.

\[ Z(\omega) = Z_{CPE}(\omega) + Z_{Cole}(\omega) = \left( \frac{1}{Q_0(j\omega)^n} \right) + \left( R_\infty + \frac{R_0-R_\infty}{1+(j\omega\tau)^\alpha} \right), \]  

(1)

where \( \omega \) is the angular frequency (rad/s), \( j \) is the unit of the imaginary number, \( R_\infty \) equals the impedance of the sample at an infinite frequency, \( R_0 \) is the impedance of the sample at frequency equal zero, \( \tau \) is the characteristic time of the sample and \( \alpha \) is a dimensionless parameter with theoretical values between 0 and 1 [11]. This equation represents the mathematical equivalent of the electric circuit shown in figure 1. Theoretical values for the capacity \( Q_0 \) and the dimensionless parameter \( n \) can also have theoretical values between 0 and 1, emphasizing that the equation is equivalent to an ideal capacitor when \( n \) is equal to 1.

3. Results
Figure 4 shows the experimental data and its simulation fitted to the proposed mathematical model for the three evaluated tissues, and as a function of degradation time (\( t = 0, 5 \) and 10 hrs). As frequency increases, simulated data does not show a suitable match with respect experimental values for fat and skin tissue. Table 1 shows the estimated Cole parameters for every tissue condition, where different dynamic range for every tissue is evident, as well as a non-linear response as a function of degradation time.
4. Discussion

In ex vivo tissue samples, as long as degradation time increases, it is expected that the cell membrane would work as a short circuit. Thus, in the initial model, CPE will be in series with the proposed resistor $R_0$, and then $R_e$ must have a similar value [11]. Nevertheless, the observation indicates that $R_0$ decreases significantly and $R_e$ remains in similar values, both when compared to their values at $t = 0$ h. Therefore, the alteration of the initial model as a function of degradation time might not be possible explained in terms of a short circuit only; it seems that a gradual disruption of the cell membrane is evident and in consequence, a gradual $t = 0$ h, $t = 5$ h $t = 10$ h muscle fat skin ionic release from the cells to the

Table 1. Estimated Cole parameters for every tissue condition

|       | $R_0$ (Ω) | $R_∞$ (Ω) | $\tau$ (s) | $\alpha$ (0-1) | $Q_0$ (Ω-1) | $n$ (0-1) |
|-------|-----------|-----------|------------|----------------|-------------|-----------|
| Muscle |           |           |            |                |             |           |
| $t = 0$ h | 602.313e6  | 70.562e6  | 0.9e-6     | 0.70           | 0.6e-6      | 0.24      |
| $t = 5$ h | 383.299e6  | 67.309e6  | 1.0e-6     | 0.61           | 0.6e-6      | 0.50      |
| $t = 10$ h | 179.172e6  | 50.519e6  | 1.0e-6     | 0.45           | 0.6e-6      | 0.24      |
| Fat    |           |           |            |                |             |           |
| $t = 0$ h | 1158.456e6 | 779.526e6 | 4.3e-6     | 0.90           | 0.6e-6      | 0.50      |
| $t = 5$ h | 955.382e6  | 802.755e6 | 70.0e-6    | 0.96           | 0.005e-6    | 0.50      |
| $t = 10$ h | 663.233e6  | 892.625e6 | 20.0e-6    | 0.59           | 0.6e-6      | 0.24      |
| Skin   |           |           |            |                |             |           |
| $t = 0$ h | 225.669e6  | 95.550e6  | 1.5e-6     | 0.75           | 0.6e-11     | 0.8       |
| $t = 5$ h | 138.780e6  | 122.516e6 | 4.0e-6     | 0.48           | 0.6e-6      | 0.5       |
| $t = 10$ h | 159.641e6  | 136.452e6 | 3.0e-6     | 0.40           | 0.6e-6      | 0.5       |

Figure 4. Nyquist plot for multifrequency bioimpedance for experimental and fitted data.
extracellular medium promote an increase in the extracellular conductivity. Thus, the extracellular resistance is significantly reduced. Such a hypothesis assumes a non-linear function of the Cole parameters through degradation progress in the three evaluated tissues, and such non-linearities might be associated with different capacities of adaptation to anaerobic metabolism, energy production, and water content under stress conditions. In principle, it seems that the mathematical model proposed and estimation of their Cole parameters to explain the tissue degradation process are not completely suitable for modeling the experimental conditions of this study. Therefore, there is no standard circuit model that may fit all tissue systems for degradation dynamic characterization, and additional RC elements should be considered accordingly to the metabolic cell tissue characteristics. Additional experiments to verify the observations are warranted.

5. Conclusion
The dynamic Cole parameters of the proposed mathematical model estimated for different degradation times in *ex vivo* muscle, skin, and fat tissues indicate non-linear degradation progress in the three evaluated tissues, and such non-linearities might be associated with different factors that modulate the cell membrane disruption. Some factors could be associated with every type of tissue and its capacities of adaptation to anaerobic metabolism, energy production, and water content under stress conditions.

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