Thesis for the degree of Licentiate of Engineering

Electrical Bioimpedance Cerebral Monitoring:
Effects of Hypoxia

by

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ELECTRICAL BIOIMPEDANCE CEREBRAL MONITORING: EFFECTS OF HYPOXIA

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Dedicado a mis abuelas:
Luisa y Teresa
Electrical bioimpedance spectroscopy is one way to study the electrical properties of biological matter. Different applications of electrical bioimpedance measurements have already been used in both research and clinical scenarios e.g. impedance plethysmography, total body water contents, etc. The electrical properties of tissue reflect the electrical characteristics of the constituent elements of the tissue and depend on its structure. Thus study of the electrical properties not only makes it possible to differentiate among tissues but also to determine the tissue condition. During hypoxia/ischemia the cell activates a certain chain of mechanisms of cellular adaptation in response to the insult. A consequence of these response mechanisms is that the biochemical composition of the cellular environment is altered and the cells swell (cellular edema). These alterations affect the electrical properties of tissue and the changes can be observed through measurement of the electrical bioimpedance of the affected tissue. Based on these ideas, this research work studies the effects of hypoxia/ischemia on the brain electrical impedance. The aim is to obtain the fundamental knowledge that may lead to the development of useful clinical tools for cerebral monitoring based on electrical bioimpedance spectroscopy.
First of all I would like to thank my supervisor Prof. Kaj Lindecrantz, not only for his contribution to the technical development of this thesis but also for his support and guideline, which have allowed me to perform the research work contained in this thesis. I honestly think that he is doing a great job because I feel very well supervised.

I also would like to mention two very special contributors to my research work and to my development as a researcher in the biomedical engineering field: Prof. Torsten Olsson and Prof. Ingemar Kjellmer, just to say that I fell very fortunate for having the opportunity to work and learn from them.

This work has been possible due to the contribution of the medical and clinical staff at Sahlgrenska University Hospital and Göteborg Universitet. Therefore, I am grateful for their cooperation that I consider essential for the existence of this thesis and for my future research work.

There are two special fellows, Nisse and Johan, who quite often help me to find the way out of my own mental mazes, where I often end up trying to understand some “basic” concepts and “important” findings of my research. Usually they are neither as basic nor as important as I first thought. Anyway thank you both.

I would like to thank my employer Högskolan I Borås for providing me with this opportunity to fulfill the dream of being a postgraduate student.
I would like to thank the people at S2 for providing such a comfortable and motivating work environment for doing research and becoming a doctor eventually.

For many of us a good latte in the morning is essential to perform a good job, I want to thank my regular coffee companions for sharing those tasteful moments. Special thanks to Ramon and Thomas for the lattes and much more.

I have a few friends who with their support and encouragement have helped me in several ways to keep on pursuing my dreams. I want to thank my friends from Linköping: Eduardo, Greger, Paco and Ramon for being that close. I want to especially thank my four brothers in Spain, you make easy to stay far away from home (in the best sense 😊). And last but not least; I want to thank Anita for always being there for me, well almost.

Among my friends in Linköping there is a special person that I want to thank because without his support, dedication and good advice I would probably not be writing this lines today. Thank you Prof. Göran Salerud.

Fernando Seoane Martinez,
Göteborg, April 2005.
Electrical bioimpedance may prove to be useful also for cerebral monitoring and the aim of this work is to explore this idea. The research activity reported in this thesis has been performed under the supervision of Prof. Kaj Lindecrantz as the main task of the research project denominated “Brain damage: Detection and localization of cell swelling”. This research project is funded by The Swedish Research Council (Vetenskapsrådet) by the research grant number 2002-5487.

The central hypothesis of this research activity is that effects in the brain tissue of the cellular swelling can be detected by measuring the changes in the transcephalic electrical bioimpedance.

This research has been performed through the collaboration between the following research and academic institutions: The School of Engineering at University College of Borås, the Department of Signals and Systems at Chalmers University of Technology, Sahlgrenska University hospital and the Sahlgrenska Academy at Göteborg Universitet.

This licenciate thesis is organized in two main parts. The first part will provide some background and an overview of the work performed. The second part is a collection of papers.

Part I is divided into 7 chapters. Chapter 1 contains an introduction to this thesis. Chapters 2, 3 and 4 contain a scientific introduction to the main fields and core topics involved in my research activity. In Chapter 5, a brief summary of the scientific
publications included in part II is presented. Chapter 6 contains the main conclusions, the final discussion and the future work. At the end of part I, the cited bibliography is referenced and a glossary of terms is also included.

Part II includes the scientific contributions and publications derived from the research activity performed, containing the core knowledge of this thesis.
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Part I
\begin{enumerate}
\item $X$ or $x$ = Physical dimensions (i.e. length, area, etc).
\item $x$ = real electrical properties (i.e. resistivity, conductivity, etc)
\item $x^*$ = complex electrical properties (i.e. impedivity, admittivity, etc)
\item $X$ = real electrical magnitudes (i.e. conductance, resistance, etc).
\item $X^*$ = complex electrical magnitudes (i.e. admittance, impedance, etc).
\item $\tan$ = trigonometric functions (i.e. tangent).
\item $j$ = the unit imaginary number $\sqrt{-1}$
\end{enumerate}
1.1 Background

Cell swelling, cellular edema, is a consequence of the physiological adaptations that the cell goes through in response to a threatening stimulus and it may be an early manifestation preceding cellular injury. If it is possible to detect the cellular edema early enough, potentially, counteractive measures can be taken and brain damage avoided.

1.1.1 Clinical Background

Cerebral hypoxia/ischemia is always a severe threat to the brain. The cause of hypoxia/ischemia can be either local as in the case of cerebral infarction (stroke) or there may be as global hypoxia due to collapsing circulation or poor oxygenation.

In the perinatal period the most common cause of brain damage in the mature fetus. Up to one out of every 250 newborn babies suffer from perinatal asphyxia in developed nations. Resulting in tens of thousands of newborns with brain damage every year worldwide. The mortality rate is as high as 50% in the severe cases. Many of the infants who survive suffer severe neurological disabilities. The incidence of long-term complications depends on the severity of the hypoxic/ischemic insult. Among the most frequent consequences are mental retardation, ataxia, epilepsy, and cerebral palsy. Conservative estimates of the yearly cost to society for treatment and care of each affected child lie around half a million € (Berger and Garnier 1999). Thus a better understanding of the praxis behind hypoxic/ischemic brain damage will have implications, not only on the health of the affected individuals, but also on worldwide economy.

The survival rates of patients with brain damage have increased markedly during the last few decades, but the number of patients with symptoms and handicaps from acquired brain damage has not decreased. Society pays for better survival rates by an increased risk of permanent neurological impairment in the new survivors. The
reduced mortality can be ascribed to a combination of new basic knowledge, improved treatments and improved possibilities for intensive care monitoring of vital functions such as circulation, respiration and metabolism. However, we lack knowledge about the damaging processes and methods to detect, prevent and treat impaired brain function before a permanent lesion develops. There is a strong demand for better understanding of the various processes in the brain, which we can influence today through medication and other means.

Both for the building of this understanding and for an improved care there is a need for efficient methods for continuous monitoring of the brain and brain function. Adequate methods may exist for the diagnosis of morphological changes that have already occurred, but there are no techniques to detect clinical situations of impending brain injury. Electrical impedance technology has been successfully applied in many clinical situations to determine physiological parameters, i.e. total body water content, respiration rate. Non-invasive measurements of bioelectrical impedance are safe and do not carry any discomfort for the patient.

1.2.2 Research Goals

Potentially, electrical bioimpedance may be developed into a non-invasive, continuously usable method for detection and localization of hazardous events of cell swelling, in the brain. The aim of this project is to investigate some basic properties of the electrical bioimpedance measured across the brain or more specifically:

a) To establish the basic characteristics of the electrical impedance across the brain, the frequency dependency in the normal as well as the hypoxic brain.

b) To verify preliminary results regarding time relation between impedance alterations/cell swelling and cerebral ischemia.

c) To design a robust instrumentation for multi-frequency bioimpedance measurements extendible to multichannel applications.

d) To investigate the influence of the skull bone on the current flow between electrodes, a factor that may hamper the ability to localize smaller areas of cell swelling. The first goal is to localize swelling in the correct hemisphere.

e) To take the first steps towards, validating the clinical value of electrical transcephalic impedance.

The scientific activities reported in this thesis deal with the research goals a) and b), as listed above.
2.1 Tissue Electrical Conduction

The electrical conductance of biological tissue is determined by its constituent elements. In essence, tissue consists of extracellular fluid and cells containing the intracellular fluid inside a cell membrane. The extracellular fluid is the medium surrounding the cells, also denominated the extracellular space. It contains proteins and electrolytes including the plasma and the interstitial fluid. The cell is composed of a lipid bilayer plasma membrane surrounding the protoplasm that contains the cytosol, the organelles and the nucleus of the cell. A general definition of living tissue is:

“A part of an organism consisting of an aggregate of similar cells and the intercellular substances surrounding them organized into a structure with a specific physiological function.”

2.1.1 Tissue Fluids as Electrolytes

In metals the electric charge carriers are electrons but in electrolytes the charge carriers are ions. An electrolyte exhibits ionic DC conductivity and it is define in the following way:
Both intracellular and extracellular fluids are electrolytes because they contain ions free to migrate and transport the electric charge. Therefore we can consider biological tissue, electrically and macroscopically, an ionic conductor. The total ionic conductivity of a solution depends on the concentration, activity, charge and mobility of all the free ions in the solution. The most important ions contributing to the ionic current in living tissue are K⁺, Na⁺ and Ca²⁺. The viscosity and temperature of the solution are also important factors influencing in the ionic conductivity.

Table 1. Approximated Concentration of Ions in Living Tissue

<table>
<thead>
<tr>
<th></th>
<th>Intracellular</th>
<th>Extracellular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>10 mEq/L</td>
<td>140 mEq/L</td>
</tr>
<tr>
<td>K⁺</td>
<td>140 mEq/L</td>
<td>4 mEq/L</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>4 mEq/L</td>
<td>103 mEq/L</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>0.0001 mM</td>
<td>2.4 mEq/L</td>
</tr>
</tbody>
</table>

* Data from (Guyton and Hall 2001)

Ionic conductance is a transfer of charges accompanied by movement of substance producing changes in the bulk of the electrolyte. This way DC ionic conductivity is linear only for a limited period of time and only when the strength of the applied external electric field is not high.

For more detailed information about electrolytes on living tissue, see Grimmes and Martinsen (2000).

2.1.2 The Plasma Membrane

The plasma membrane completely surrounds the cell. It is a thin and elastic structure 75 to 100 Å thick and it is primarily composed of proteins (≈55%) and lipids (≈43%).

The Bilayer of Lipids Structure

The elemental structure of the plasma membrane is a double layer formed by only two lipid molecules. The lipid molecules forming the bilayer have a hydrophilic side and a hydrophobic side; the hydrophobic sides attract each other. This attraction forces the hydrophilic head into the exterior of the structure and the hydrophobic into the interior. This structure is continuously replicated in every direction creating the plasma membrane. See Figure 2.

The intrinsic electric conductance of this structure is very poor and it is considered as a dielectric. The total structure formed by the intracellular fluid, plasma membrane and extracellular fluid, i.e. a conductor-dielectric-conductor structure, behaves as a capacitor with an approximate capacitance of 0.01 F/m².

The Transmembrane Channels

Intermixed with the lipid bilayer structure there are proteins of various types. One of them is integral protein. This type of protein is inserted in the lipid bilayer
crossing through the plasma membrane, creating very narrow channels for substances to pass through the plasma membrane like ions and water. From an electric point of view, these channels allow current to pass through the insulating membrane in a passive manner.

![Figure 2. The lipid bilayer structure of the plasma membrane](image)

### 2.1.3 Tissue and Dielectricity

Any material with the ability to store capacitive energy can be classified as a dielectric and living tissue has this ability due to its constituents at any level, molecular, subcellular, or cellular.

The composition of the extracellular and the intracellular fluid, especially the organelles, contributes to the overall behavior of tissue as a dielectric, but the plasma membrane is the cellular structure with the major contribution to the dielectric behavior of living tissue. The dielectric properties are also influenced by the specific tissue structure.

The electrical properties of biological tissue are due to relaxation phenomena and a number of different theories have been applied on biological matter to explain the responsible relaxation mechanisms: general relaxation, structural relaxation and polar relaxation. The combined effects of the different polarization mechanisms are responsible for the observed electrical properties throughout the complete frequency range. Extensive reviews of the dielectric properties of tissues, including developed theories, can be found elsewhere (Schwan 1957; Foster and Schwan 1989, 1994).

**Dielectric Theory: Basic Concepts and Definitions**

Usually, dielectricity theory is explained with the help of the concept of the capacitor. In a capacitor, the passive electrical properties of the dielectric material held between the two plane-parallel electrodes are completely characterized by the experimentally measured electrical capacitance $C$ and conductance $G$. In the case of a uniform field, the capacitance is defined in equation (1) and measured in Farads (F). The conductance is defined in equation (2) and measured in Siemens (S):

\[
C = \frac{\varepsilon_r \times \varepsilon_0 \times A}{d} \quad \text{Equation (1)}
\]

\[
G = \frac{\sigma \times A}{d} \quad \text{Equation (2)}
\]
Chapter 2. Electrical Properties of Living Cells and Tissue

$A$ is the area of the plane electrodes and $d$ is the electrode separation distance. See Figure 3(a).

![Figure 3. (a) A Capacitor: dielectric material between two metallic surfaces. (b) The equivalent circuit with a capacitance in parallel with a conductive element](image)

$\sigma$ denotes the electrical conductivity of the material; it represents the current density induced in response to an applied electric field and it indicates the facility of the charge carriers to move through the material under the influence of the electric field. In the case of living tissue, the conductivity arises mainly from the mobility of the extracellular and intracellular ions.

$\varepsilon_0$ denotes the dielectric permittivity of vacuum, and its constant value is $8.854 \times 10^{-12}$ F/m, whilst $\varepsilon_r$ denotes the permittivity of the material relative to $\varepsilon_0$. The permittivity reflects the extent to which charge distributions within the material can be distorted or polarized in response to an applied electric field. In the case of biological tissue, charges are mainly associated with electrical double-layer structures, i.e. plasma membrane, around solvated macromolecules and with polar molecules, i.e. molecules that have a permanent electric dipole moment.

**Admittance-Impedance and Conductivity-Resistivity**

Some electrical properties of the circuit in Figure 3(b) depend on frequency and there are several ways to describe this. In the case of sinusoidal oscillating field with natural frequency $\omega$, the complex admittance $Y^*$ can be expressed

$$Y^* = G + j \omega C = \left(\frac{A}{d}\right)(\sigma + j \omega C \varepsilon_r \varepsilon_0)$$

Equation (3)

from which the complex conductivity $\sigma^*$, also called admittivity or specific admittance, is defined:

$$\sigma^* = \sigma + j \omega \varepsilon_r \varepsilon_0$$

Equation (4)

The impedance is defined as the inverse of the complex admittance and can be written as

$$Z^* = \frac{1}{Y^*} = R + jX = \frac{G - j \omega C}{G^2 + (\omega C)^2}$$

Equation (5)
from which the complex specific impedance $z^*$, also denominated impedivity, of the material is defined:

$$z^* = \frac{1}{\sigma^*} = \frac{\sigma - j\omega \varepsilon_0 \varepsilon_r}{\sigma^2 + (\omega \varepsilon_0 \varepsilon_r)^2}$$

Equation (6)

Note that the complex specific impedance $z^*$ also denominated complex resistivity and denoted by $\rho^*$, is the inverse of the complex conductivity $\sigma^*$ (Grimmes and Martinsen 2000) and its unit is $\Omega\text{m}$.

### 2.1.4 Frequency Dependency. The Dispersion Windows

Living tissue is considered as a dispersive medium, both permittivity and conductivity are functions of frequency. See Figure 4. This observed frequency dependence is denominated dispersion and it arises from several mechanisms (Foster and Schwan 1994). Schwan (1957) identified and termed three major dispersions: $\alpha$, $\beta$, and $\gamma$-dispersions. Another subsidiary dispersion was noted for the first time in 1948 (Rajewsky and Schwan 1948) and later identified and termed $\delta$-dispersion (Schwan 1994). See Figure 4.

**$\alpha$-dispersion**

The understanding of the $\alpha$-dispersion remains incomplete (Schwan 1994). A multitude of various mechanisms and elements contribute to this frequency dependence. Three well established factors are presented below (Schwan and Takashima 1993):

- The frequency dependent conductance of the channel proteins present in the cell membrane.
- The frequency dependency of the surface conductance and capacitance, largely caused by the effect of the counterion atmosphere existing near charged cell surfaces.
- The effect of the endoplasmic reticulum, when it exists.
The $\beta$-dispersion is caused mostly by the cellular structures of tissue, due to the low conductivity of the plasma membrane of the cells forming the tissue. It takes time to charge the membranes through the conducting mediums, the extracellular and intracellular fluids. The introduced time constant is determined by the plasma membrane capacitance, the cell radius and the fluid conductivities (Schwan 1957).

There are other tissue constituents contributing to the $\beta$-dispersion caused by the cell structure as well (Foster and Schwan 1989), e.g. proteins, aminoacid residues and organelles. For a more detailed description of the contributions of the different tissue constituents to the different dispersions, the reading of the article review (Pethig and Kell 1987) is suggested.

The $\gamma$-dispersion

This frequency dependence is caused by the high content of water in cells and tissues. Tissue water is identical to normal water, which relaxes at 20 GHz, except for the presence of proteins and aminoacids, etc. Tissue water displays a broad spectrum of dispersion from about 100 MHz to some GHz.

The $\delta$-dispersion

It is a weak subsidiary dispersion effect due to protein bound water. It is observed around 100 MHz.

<table>
<thead>
<tr>
<th>Contributing Biomaterial Element</th>
<th>Dispersion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\alpha$</td>
</tr>
<tr>
<td><strong>Water and Electrolytes</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Biological Macromolecules</strong></td>
<td></td>
</tr>
<tr>
<td>Amino acids</td>
<td>$X$</td>
</tr>
<tr>
<td>Proteins</td>
<td>$X$</td>
</tr>
<tr>
<td>Nucleic acids</td>
<td>$X$</td>
</tr>
<tr>
<td><strong>Vesicles</strong></td>
<td></td>
</tr>
<tr>
<td>Surface Charge</td>
<td>$X$</td>
</tr>
<tr>
<td>No Surface Charge</td>
<td>$X$</td>
</tr>
<tr>
<td><strong>Cells with Membrane</strong></td>
<td></td>
</tr>
<tr>
<td>+ Fluids free of protein</td>
<td>$X$</td>
</tr>
<tr>
<td>+ Tubular system</td>
<td>$X$</td>
</tr>
<tr>
<td>+ Surface charge</td>
<td>$X$</td>
</tr>
<tr>
<td>+ Membrane relaxation</td>
<td>$X$</td>
</tr>
<tr>
<td>+ Organelles</td>
<td>$X$</td>
</tr>
<tr>
<td>+ Protein</td>
<td>$X$</td>
</tr>
</tbody>
</table>

* Table contents from Schwan (1994)
3.1 Cell Electrical Conductance

The electrical characteristics of biological tissues reflect the characteristics of the constitutive cells. It is useful to look up equivalent electric circuit models of the cells and tissue, because they help to understand the conductance phenomena and to attribute a physical interpretation to the impedance parameters in biological material.

3.1.1 Electrical Circuit of the Cell

If we apply the theory of electric circuits and simplify, it is possible to deduce a simple electrical model for the cell, considering the main constituents of the cell, introduced in the previous chapter. See Figure 7.

If current is injected into the extracellular medium, it can do the following things:

a) Flow around the cell through the extracellular fluid. $R_e$ in Figure 7(a) represents the corresponding resistance.

b) Flow through the cell across the plasma membrane. In Figure 7(a), $C_m$ represents the capacitance and $R_m$ the resistance of the plasma membrane. $R_m$ is mainly the result of the transmembrane ionic channels and $R_i$ represents the resistance of the intracellular medium.

In practice the value of $R_m$ is very high. At low frequencies, near DC, the current is not able to penetrate the cell and most of the current flows around the cell. But with increasing frequency the capacitance of the plasma membrane allows current to cross and a portion of the current is able to flow through the cell. At frequency above 1 MHz the membrane capacitance is not an impediment of the current and it flows indiscriminately. At frequencies above 1 MHz, the membrane capacitance is not an impediment to the current and it flows indiscriminately through the intra- and extracellular medium.
Chapter 3. Bioelectrical Impedance

Figure 7. Equivalent electrical circuit of a cell. The circuit in b) is the equivalent of the model in a) after performing some circuit simplifications. The circuit in c) is the equivalent circuit of the cell neglecting the effect of $R_m$. Note that $C_m/2$ is replaced by $C_m^*$. Usually, as the membrane conductance is very low, the effect of $R_m$ is neglected simplifying the equivalent electric circuit. In this case, the model only accounts for a single dispersion; the frequency dependence introduced by the capacitor $C_m$. See Figure 7(c). The use of this simplified model is widely spread and it is appropriate in the broad range from hundreds of Hertz to a few tens of MHz.

3.2 Tissue Conductivity, Resistivity and Impedance

Biological tissue is built up by cells, and therefore, from an electrical point of view, it can be considered as an aggregation of conductive cells, suspended in a conductive fluid.

3.2.1 The Model of Suspension of Spherical Cells

It is an old established practice in biophysical studies to consider biological tissue as a suspension of spherical cells. At first, this model was believed to be reliable only when the concentration of cells was considered to be small (Maxwell 1891) but experimental measurements suggest that the model is reliable for high concentration as well, up to concentrations nearly 80% (Cole et al. 1969; Pavlin et al. 2002).

Figure 8. Current paths in a Suspension of Cells

*Electrical Resistivity of a Spherical Cell*

Consider a cell as a sphere of radius $a_2$ containing a conductive medium of resistivity $r_2$ surrounding a spherical and centered core of radius $a_1$ and resistivity $r_1$, we
can replace the set of the two concentric spheres by a single sphere of radius \( a_2 \) and uniform resistivity, \( r \), as given in the equation (7), from article 313 of Maxwell’s treatise (Maxwell 1891). See Figure 9.

\[
\frac{r}{(2 r_1 + r_2) a_2^3 + (r_1 - r_2) a_1^3}{2 (r_1 + r_2) a_2^3 - 2 (r_1 - r_2) a_1^3} = \frac{r}{r_2}
\]

Equation (7)

![Figure 9](image)

Figure 9. The conductance of a stratified sphere of radius \( a_2 \) containing a conductive medium of resistivity \( r_2 \) and a centred solid sphere of radius \( a_1 \) and resistivity \( r_1 \) is equivalent to a single sphere of radius \( a_2 \) containing a conductive medium of resistivity \( r \) given by equation (7).

In the case of cells, the plasma membrane is very thin, \( a_2 - a_1 = \delta \), and the resistivity of the membrane is very high compared with the resistivity of the intracellular fluid, \( r_2 \gg r_1 \). The equivalent resistivity \( r \) can be simplified (Cole 1928).

First simplification, using \( a_2 - \delta = a_1 = a - \delta \) and \( a \gg \delta \):

\[
r = \frac{r_1 + \frac{\delta r_2}{a}}{l - \frac{2 \delta}{a}} \left( l - \frac{r_1}{r_2} \right)
\]

Equation (8)

Note that \( a_2 \) is substituted by \( a \) and terms multiplying higher powers than 1 of \( \delta \) are neglected.

Second simplification, \( r_2 \gg r_1 \Rightarrow l \gg \frac{r_1}{r_2} \):

\[
r = r_1 + \frac{\delta r_2}{a}
\]

Equation (9)

Considering that the plasma membrane introduces a capacitive effect, \( \delta r_2 \) is substituted by \( z \text{*}_m \) denoting the impedivity of the membrane for a unit area in \( \Omega \text{cm}^2 \) including the reactive component (Cole 1968b).

\[
r = r_1 + \frac{z \text{*}_m}{a}
\]

Equation (10)
Chapter 3. Bioelectrical Impedance

In (Cole 1928, 1932) a constant phase angle $\rho_m$ for $z_m^*$ is assumed, in such a way that $\tan (\rho_m) = \frac{x_m}{r_m}$. This implies that if $z_m^* = r_m + jx_m$ then $r_m = k x_m$, where $k = \cot (\rho_m)$. This assumption fits the empirical data but it lacks a theoretical demonstration.

$$\tan \rho_m = \frac{x_m}{r_m} \rightarrow z_m^* = r_m + jx_m \iff z_m^* = |z_m| \angle \rho_m$$  \text{Equation (11)}

**Electrical Resistivity of a Suspension of Spherical Cells**

The equivalent electrical resistivity of a sphere containing a uniform suspension of spheres of radius $a$, and resistivity $r_i$, in a medium with a resistivity $r_e$ and radius $a_2$ was calculated by Maxwell in the article 314 (Maxwell 1891).

$$r = r_e \frac{(l-f)r_e + (2+f)r_i}{(l+2f)r_e + 2(l-f)r_i}$$  \text{Equation (12)}

The average resistivity of the suspension is given by $r$ in equation (12), where $r_e$ is the resistivity of medium surrounding the spheres, $r_i$ is the resistivity of the contained spheres and $f$ is the volume factor of concentration of cells (the units of the resistivities $r_e$, $r_i$ and $r_f$ are in $\Omega \text{cm}$ and the volume factor $f$ is dimensionless). Equation (12) is re-arranged as in (Cole 1928).

**Figure 10.** The conductance of a sphere of radius $a$, containing a conductive fluid of resistivity $r_e$ in which there are uniformly disseminated solid spheres of radius $a$ and resistivity $r_i$ is equivalent to a single sphere of radius $a_2$ containing a conductive medium of resistivity $r$ given by equation (12).

**Figure 11.** A sphere containing conductive spherical cells of radius $a$ disseminated in a conductive fluid.
Chapter 3. Bioelectrical Impedance

To substitute the internal spheres by spherical cells, \( r_i \) in equation (12) is substituted by \( r \) from equation (10) and the specific complex impedance \( z^* \) of the suspension containing spherical cells is given by equation (13) (Cole 1968b). Note that the notation changes from \( r_i \) to \( r_i \) and from \( r \) to \( z^* \).

\[
\begin{align*}
\quad z^* &= r_e \frac{(l - f) r_e + (2 + f) \left( r_i + \frac{z_m^*}{a} \right)}{(l + 2 f) r_e + 2(l - f) \left( r_i + \frac{z_m^*}{a} \right)} \\
\end{align*}
\]

Equation (13)

Usually the resistivity effect of the plasma membrane is neglected and the membrane is considered as an ideal capacitor. Thus the specific impedance of the membrane is only imaginary \( z_{m}^* = j \omega \mu m \), with the reactive part

\[
x_m = -\frac{l}{\omega \mu m}
\]

Equation (14)

3.2.2 Tissue Impedance

The resistivity is an intrinsic parameter of the materials independent of the shape and therefore the resistivity given by equation (13) is the resistivity of any compound medium consisting of a substance of resistivity \( r_e \) in which there are disseminated spheres of radius \( a \) and resistivity \( r_i \) with a shell of impedivity \( z_{m}^* \).

In the simple case of a pure resistive cylindrical conductor, as in Figure 12, the total resistance \( R \) of the conductor is given by equation (15),

\[
R = \rho \frac{L}{S}
\]

Equation (15)

\( L \) is the length of the cylinder, \( S \) is the cross-sectional area of the conductor and \( \rho \) is the resistivity of the material.

\[\text{Figure 12. Cylindrical conductor with real resistivity } \rho, \text{ length } L \text{ and cross-sectional area } S\]
4.1 Introduction to Cellular Damage

The cell is usually confined to a narrow range of functions. This function specificity of the cell is partly result of its genetic program and partly due to the surrounding environment. Access the energy and the metabolic capacity of the cell play important roles as well. The state of the cell handling normal physiological demands is denominated homeostatic steady state. In the presence of a pathological stimulus or excessive physiological stress, the cell has the capacity to adapt itself, achieving a new, but altered steady state to preserve the viability of the cell. This process is denominated cellular adaptation and when the limits of the adaptability of the cell are exceeded cell injury occurs. Depending on the severity and the duration of the stimuli, cell injury is reversible up to a certain point after which irreversible cell injury occurs, leading to cell death. The capacity of the cellular adaptation varies among different types of tissues, e.g. brain tissue exhibits a very high sensitivity to hypoxic insults (Cotran et al. 1989; Guyton and Hall 2001).

4.2 Hypoxic/Ischemic Cellular Damage

One of the most important and common causes of cell injury is hypoxia; it strikes at one of the most vulnerable intracellular systems, namely the aerobic oxidative respiration mechanism of the cell, involving oxidative phosphorylation and production of ATP. Among the causes of hypoxia the most common is ischemia, i.e. loss of blood supply. Other common causes of hypoxia are inadequate oxygenation of the blood after a cardiorespiratory failure or loss of the oxygen-carrying capacity of the blood like in anemia.
4.2.1. Ischemic/Hypoxic injury mechanism

In the cell, the structural and biochemical elements are strongly linked and a strike against one system leads to a widespread and quick chain of events affecting other systems in the cell. The duration and severity of the pathological stimulus are important factors for the severity of cell injury, but the type of cell and its current state and adaptability also strongly influence the final outcome; e.g. a similar hypoxic insult injures more severely brain tissue than muscle tissue.

Hypoxia is simply a reduction in the availability of oxygen while ischemia is a reduction in the blood flow. In ischemia, in addition to the lack of oxygen, there is a reduction in the delivery of metabolic nutrients and an excessive accumulation of catabolites, otherwise removed by the blood flow. Therefore, ischemia usually leads to injure tissues faster than hypoxia.

Cellular Adaptation

In a hypoxic/ischemic situation, the cell adapts itself to the lack of available oxygen. The cell stops the generation of ATP with the use of oxygen and changes from aerobic to anaerobic metabolism, producing ATP from glycogen and creatine phosphate instead. Anaerobic metabolism results in the accumulation of osmotic active products like lactic acid and inorganic phosphates, causing a reduction in the intracellular pH value and influencing the intracellular osmotic pressure.

The availability of ATP is severely reduced and the energy-dependent Na⁺/K⁺ pump in the plasma membrane reduces its transport activity or loss it completely. The cell is then no longer able to keep the ionic gradients across the membrane. The failure of this active transport results on alteration in the intracellular ionic contents (Hansen 1984; Cotran et al. 1989; De Haan and Hasaart 1995; Berger and Garnier 1999; Yi et al. 2003). Na⁺ increases and K⁺ decreases, resulting in a membrane depolarization.
Chapter 4. Hypoxic/Ischemic Brain Damage

(Hansen 1985). In the absence of a membrane potential, Cl⁻ ions (Berger and Garnier 1999) and large amounts of Ca²⁺ (Hansen 1984; De Haan and Hasaart 1995) flow through the voltage-dependent ion channel into the cell.

The combined effect of the process mentioned, the failure of the active transport, the opening of the voltage-dependent channels and the anaerobic metabolism, produces an abnormally high intracellular concentration of catabolites and ions. The net gain of solute induces an influx of water following the osmotic gradient, aiming to establish an isosmotic pressure on both sides of the plasma membrane. Consequently, the cell swells, causing cytotoxic edema (Klatzo 1994; De Haan and Hasaart 1995), one of the earliest and most common histological manifestations of hypoxic injury (Cotran et al. 1989), also denominated cellular edema or acute cell swelling.

Figure 14. Biochemical and physiological processes during hypoxia in the cell.

At the same time as the water influx, the endoplasmic reticulum suffers an early dilation followed by a detachment of the ribosomes from the granular endoplasmic reticulum. If hypoxia persists, other alterations take place like blebs formation on the surface of the cell and mitochondrial swelling.

Reversible and Irreversible Cell Injury

All the previously mentioned cellular alterations are reversible if normoxia is re-established; the cell is in a state of reversible cell injury. If the insult continues, the cell reaches “the point of no return” and irreversible cell injury ensues. There is no generally accepted explanation to the key biochemical mechanisms behind transition from reversible to irreversible cell injury. However, in certain ischemic tissues, certain
structural and functional changes indicate that the cells have been irreversibly injured (Kumar 2005).

Types of Cell Death: Necrosis and Apoptosis

There are two identified types of cell death: necrosis and apoptosis. Necrosis is always a pathological process, while apoptosis may not need to be associated with cell injury. Cell death in the hypoxic/ischemic injury mechanism occurs mainly by necrosis. In brief, the above-mentioned changes in intracellular pH value and ionic concentration damage the membranes of the lysosomes. Hydrolytic enzymes are released into the cytoplasm and trigger a chain of events, resulting in necrosis. The cell is eventually dissolved in the extracellular fluid (De Haan and Hasaart 1995; Berger and Garnier 1999). For a more detailed description of the cell death mechanisms see (Kumar 2005).

4.2.2 Ischemia-Reperfusion Injury Mechanism

There is one injury mechanism closely related to the hypoxic/ischemic injury mechanism, the ischemia-reperfusion mechanism. When the normal oxygen level and blood flow are restored, the cells will recover from the injury, provided that the cells were reversibly injured. If the cells were irreversibly injured, new injurious processes started during reperfusion, resulting in cell death through necrosis, as well as apoptosis, of cells that otherwise could have recovered. This mechanism is of special interest to us for two main reasons:

- It occurs in most of the hypoxic/ischemic injury cases.
- It can be medically treated, thereby reducing its damaging effects.
4.3 Hypoxia in the brain

As was pointed out above, different types of cells and tissues react in different way to hypoxia; brain tissue is especially vulnerable to lack of oxygen (Bramlett and Dietrich 2004). To function normally, the brain needs oxygen but it also requires sufficient supply of glucose (Berger and Garnier 1999). The transmission of electric impulses and biosynthetic reactions within the neurons continuously require an intracellular source of energy. This energy is usually produced by the breakdown of glucose during the aerobic glycolysis. When the cell resorts to anaerobic glycolysis during hypoxia, the availability of intracellular glucose is drastically reduced. Thus neurons are much more susceptible to hypoxia than most types of cells, suffering ischemic necrosis after only a few minutes after the hypoxic insult (Cotran et al. 1989).

4.3.1 Hypoxia and Perinatal Asphyxia

It has been observed the perinatal brain exhibits a higher robustness against hypoxic/ischemic insults than the adult brain. There are several reasons for this special robustness. To begin with the amount of synapses in the perinatal brain is much smaller than in the adult brain, consequently the oxygen demand is much lower. Also due to the particular risk associated with bird the perinatal brain has a special protection mechanism against hypoxia/ischemia. Before the oxygen supply has been reduced too much, the blood flow is redistributed to maintain enough oxygen available to maintain the aerobic cerebral metabolism. The augmentation of cerebral blood flow is done at the expense of the perfusion to other organs and systems like muscles, skin, and kidneys. If blood flow redistribution does not satisfy the oxygen requirements, the brain cells resort to anaerobic metabolism and the chain of events will develop as explained above (De Haan and Hasaart 1995).

4.4 Hypoxia and Impedance

As we have seen in previous sections, the electrical properties of biological tissue depend on the biochemical composition and its structure. During hypoxia/ischemia, the cells and the tissue they build up, adapts and suffer modification in their composition, size and form.

During the cellular adaptation and the reversible injury phase, the ionic redistribution in the cellular environment, the accumulation of catabolites in the intracellular space, the cell swelling and the consequent shrinking of the extracellular space modify the conductivity of the intracellular and extracellular fluids, affecting the total impedance of the tissue. During the irreversible injury and cell death phase the destruction of the membranes of the organelles and ultimately the plasma membrane change completely the electrical properties of the tissue.
5. Papers

This licentiate report contains four papers: one submitted journal paper and three presented conference contributions. All the papers deal with the effects of hypoxic/ischemic cell swelling on the electrical bioimpedance of the brain.

**Paper A. Brain Electrical Impedance at various Frequencies: the Effect of Hypoxia**

This paper was presented in the poster session 3.7.1. of the 26th annual international conference, EMBC04, hosted by the IEEE Engineering in Medicine and Biology Society and held in San Francisco, California, in September 2004 and it is published in the conference proceedings.

In this work, a piglet model is used to measure the effect of hypoxia on the electrical impedance of the brain. The impedance is measured using the four-terminal method over a broad frequency range, from 20 kHz to 750 kHz, and the effect of 45 minutes of deprivation of oxygen is studied on both real and imaginary parts of the impedance. The main contributions of this work are the following:

- Changes in the electrical impedance of the brain, both the resistive and the reactive part, are confirmed during hypoxia.

- It is shown that the observed changes are frequency dependent. The changes in resistance are bigger than those in reactance at low frequencies and smaller at higher.

**Paper B. Bioelectrical Impedance during Hypoxic Cell Swelling: Modelling of Tissue as a Suspension of Cells**

This paper was presented in poster session II of the 12th International Conference on Electrical Bio-Impedance, ICEBI’04, hosted by the International
Chapter 5. Included Papers

Society for Electrical Bio-Impedance and held in Gdansk, Poland in June 2004. the paper is published in the conference proceedings in the section Cells & Cultures & Tissues.

In this work, the cell swelling effect of hypoxia on the electrical impedance of tissue is theoretically modeled as a suspension of cells with increasing radius. In the numerical calculation of the equivalent specific resistance, the plasma membrane is considered as an ideal capacitor and a two-dimensional approach is used to calculate the impedance of the conductor. The numerical results are related with experimental measurements performed on the brain of asphyxiated piglets and there is an agreement between the empirical data and numerical data, especially in the frequency dependency of the reactive and resistive part. The main contribution of this work is the following:

- The model of the suspension of cells is demonstrated to be a suitable model for understanding the changes of the electrical impedance of the brain during cell swelling.

**Paper C. Evolution of Cerebral Bioelectrical Resistance at various Frequencies during Hypoxia in Fetal Sheep**

This extended abstract was presented in the poster session of the annual conference of Engineering and Physical Sciences in Medicine, EPSM2004, hosted by the Australasian College of Physical Scientists and Engineers in Medicine and held in Geelong, Australia, in November 2004. The paper is published in the conference proceedings.

In this work, the effect of ischemia on the cerebral electrical resistance of fetal sheep is studied. Ischemia is induced during 25 minutes and resistance is measured simultaneously at two frequencies, 20 kHz and 300 kHz. The time evolution at both frequencies is observed and compared, with special consideration of the maximum changes and time dynamics. The main contribution of this work is the following:

- The close time relationship between the changes in the resistance of the brain and the onset of the ischemic insult is demonstrated.
- The view that measurements of resistance at low frequencies, 30 kHz, may be a better indicator than measurements at higher, 200 kHz, is strengthened.

**Paper D. Spectroscopy Study of the Dynamics of the Transencephalic Electrical Impedance in the Perinatal Brain during Hypoxia**

This paper has been submitted to the journal *Physics in Medicine and Biology*, published by the Institute of Physics.

In this work a piglet model, is used to measure the effect of hypoxia in the electrical impedance of the brain. The impedance is measured using the four-terminal method over a broad frequency range, from 20 kHz to 750 kHz, and the effect of 45 minutes of oxygen deprivation is studied on both real and imaginary parts of the impedance. The main contribution of this work is the following:
o The high frequency dependency of the changes in resistance and reactance during cell swelling is shown.

o The close time relationship between the changes in the electrical impedance of the brain and the onset of the hypoxic insult is confirmed.

o The changes in the measured impedance show a more stable sensitivity regarding frequency to cell swelling in the reactance than in the resistance.

o The reactance increases relatively more than the resistance during cell swelling.

o During cell swelling, the resistance changes the most at low frequencies and the reactance changes the most at medium-high frequencies.
6.1 Discussion & Conclusion

One consequence of hypoxia/ischemia is changes in the electrical properties of biological tissue change. These changes can be measure by means of electrical impedance spectroscopy. In the case of global hypoxia in the perinatal brain both components of the electrical impedance, resistance and reactance, change notably.

The resistance and reactance of tissue change during the cellular response to a hypoxic/ischemic insult and the observed changes are frequency dependent. This frequency dependency means that the change in resistance is more noticeable at low frequencies, when it increases the most. At intermediate frequencies, it also increases but not as much as it does at low frequency, and at high frequencies it decreases instead. In the case of the reactance, it increases at any measured frequency and the changes show a high sensitivity at medium frequencies.

In the reported studies we have used intracranial electrodes. For electrical bioimpedance to become a useful clinical tool it will be necessary to use external electrodes. In the case of non-invasive measurements the high resistivity of the skull and the low resistivity of the scalp may affect the impedance measurements significantly.

Researchers have published data regarding the effect of the skull and scalp on the measurements of resistance during hypoxia/ischemia at low frequencies, (Holder 1992a; Lingwood et al. 2002b). They have reported that the observed increment is smaller, up to 5-10 times smaller, than the observed increment in invasive measurements. In the case of the effect of the skull and scalp in the distribution of the current across the head, numerical calculations suggest that more than 80% of the current at 100 kHz can penetrate the skull and go through the brain when the current injecting electrodes are placed diametrically opposite each other (Murray 1981). Regarding reactance at any frequency or resistance at medium-high or high frequencies, the effect of the skull and the scalp remains uncertain.
6.2 Future Work

It is desirable to perform a more detailed and extended in-vivo spectroscopy study in order to evaluate the sensitivity of the variations in the impedance, and their frequency dependency. For that purpose the development a multi-frequency impedance-meter is in progress.

The studies performed have used an animal model of global severe hypoxia/ischemia, which means that the deprivation of oxygen was drastic and lasted for a long time. To get a better idea of the effects of milder degrees of asphyxia on the impedance of the brain would allow us to identify the potential areas of application.

Due to the association with the hypoxic injury mechanism and the potential opportunity for medical treatment it would very interesting to study the ischemic reperfusion injury mechanism in the future. In this case, the animal experiments will necessarily last longer, even days, and the study will especially observe the dynamics of the electrical impedance during the reperfusion phase after the ischemic insult.

As was mentioned in the conclusion, the high resistivity of the skull and low resistivity of the scalp will affect the measurements of the impedance. A non-invasive spectroscopy study is inevitably necessary to determine the clinical feasibility of the application of electrical bioimpedance technology for the detection of hypoxic/ischemic cell swelling in the perinatal brain.

In the case of performing non-invasive measurements, the high resistivity of the skull will be a very important factor. The total impedance of the skull depends on the thickness and the bone formation development, among other factors. In newborns the bone of the skull is not completely formed; the thickness is much smaller than in adults. In addition to the difference in thickness, the skull in the newborn is not completely closed yet and the fontanelle membrane is present. Under these circumstances the placement of the electrodes may be of extreme importance in terms of sensitivity. The optimum placement of electrodes is a matter that must be investigated.

In the case of detection of local hypoxia/ischemia, a localization method will be required. The method will probably need electrical bioimpedance measurements from more than one channel; therefore multichannel impedance instrumentation is necessary. Work dealing with a localization method will have to study the placement of electrodes as well the difference effects of the skull bone on impedance measurements in the newborn, child and adult.

As the ultimate aim of this project is to deliver clinically useful results, a feasibility study to evaluate the clinical value of the application of electrical bioimpedance in the monitoring of brain damage is necessary.
References


References


(Klatzo I 1994). Evolution of brain edema concepts Acta Neurochir Suppl 60 3-6


(Pavlin M, Slivnik T and Miklavcic D 2002). Effective conductivity of cell suspensions Biomedical Engineering, IEEE Transactions on 49 (1): 77-80

(Pethig R and Kell B D 1987). The passive electrical properties of biological systems: their significance in physiology, biophysics and biotechnology Physics in medicine and biology 32 (8): 933-70

(Rajewsky B and Schwan H P 1948). The dielectric constant and conductivity of blood at ultrahigh frequencies Naturwissenschaften 35 315

(Schwan H P 1957). Electrical properties of tissue and cell suspensions Adv Biol Med Phys 5 147-209


<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>Apoptosis</td>
<td>A pathway of cell death that is internally induced by a tightly regulated intracellular program allowing the activation of enzymes that degrade the cells' own nuclear DNA and nuclear and cytoplasmic proteins</td>
</tr>
<tr>
<td>Asphyxia</td>
<td>A condition caused by the inadequate intake of oxygen.</td>
</tr>
<tr>
<td>Capacitance</td>
<td>in electricity, capability of a material, body or circuit for storing electric charge</td>
</tr>
<tr>
<td>Conductance</td>
<td>The characteristic that describes the availability and the mobility of conduction charges within a material, its measure in Siemens, S. It is reciprocal of the resistance</td>
</tr>
<tr>
<td>Conductivity</td>
<td>It is a measure of the ability of a material to conduct electrical current and it is measured in Siemens per meter S/m. It is the reciprocal of the resistivity</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>The semi-fluid substance contained within the plasma membrane of the cell. It includes the organelles and surrounds the nucleus</td>
</tr>
<tr>
<td>Cytosol</td>
<td>The internal fluid of the cell excluding the organelles</td>
</tr>
<tr>
<td>DC</td>
<td>An acronym for direct current, which is a constant current with no variation over time as a result of a constant electric field</td>
</tr>
<tr>
<td>Dielectric:</td>
<td>A medium that exhibits negligible or no electrical conductivity and thus acts as a good electrical insulator</td>
</tr>
<tr>
<td>Dispersion</td>
<td>The frequency dependence of the electrical permittivity</td>
</tr>
<tr>
<td>Dispersive medium</td>
<td>A medium for which the electrical permittivity or the magnetic permeability (or both) are frequency dependent</td>
</tr>
<tr>
<td>Electrolytes</td>
<td>A substance that dissociates into ions in aqueous solution and is able to conduct electric current</td>
</tr>
<tr>
<td>EMBC</td>
<td>Engineering in Medicine and Biology Conference</td>
</tr>
<tr>
<td>Extracellular fluid</td>
<td>The volume of body fluid excluding the fluid inside the cells. It includes the plasma and interstitial fluid</td>
</tr>
<tr>
<td>Glycolysis</td>
<td>The metabolic process that breaks down carbohydrates to either pyruvic acid or lactic acid and release energy for the body in the form of ATP</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>---------------------</td>
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</tr>
<tr>
<td>Hydrophilic</td>
<td>Having a strong affinity for water; tending to dissolve in water. The opposite of hydrophilic. A characteristic of polar molecules</td>
</tr>
<tr>
<td>Hydrophobic</td>
<td>Lacking affinity for water, tending to repeal water. The opposite of hydrophilic. A characteristic of non-polar molecules</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>Reduction of oxygen supply to tissue below physiological level despite adequate perfusion of the tissue by blood</td>
</tr>
<tr>
<td>ICEBI</td>
<td>International Conference in Electrical Bio-Impedance</td>
</tr>
<tr>
<td>IEEE</td>
<td>Institute of Electrical Engineers</td>
</tr>
<tr>
<td>Interstitial Fluid</td>
<td>Part of the extracellular fluid that bathes the cells of most tissues, providing a means of delivering materials and removal of metabolic waste to and from the cells.</td>
</tr>
<tr>
<td>Intracellular fluid</td>
<td>The volume of fluid that is inside the cells</td>
</tr>
<tr>
<td>Ions</td>
<td>A particle that is electrically charged (positive or negative)</td>
</tr>
<tr>
<td>Ischemia</td>
<td>A low oxygen state usually due to obstruction of the arterial blood supply or inadequate blood flow leading to hypoxia in the tissue</td>
</tr>
<tr>
<td>Necrosis</td>
<td>Refers to a spectrum of morphologic changes accompanying cell death tissue, largely resulting from the progressive degradative action of enzymes on the exogenous lethally injured cell</td>
</tr>
<tr>
<td>Organelles</td>
<td>One of several structures with specialized functions, suspended in the cytoplasm</td>
</tr>
<tr>
<td>Perinatal</td>
<td>Pertaining to the period immediately before and after birth. The perinatal period is defined in diverse ways. Depending on the definition, it starts at the 20th to 28th week of gestation and ends 1 to 4 weeks after birth</td>
</tr>
<tr>
<td>pH</td>
<td>A logarithmic scale used to describe the relative acidity or alkalinity of a solution, measuring the concentration of hydrogen and hydroxide ions in the solution.</td>
</tr>
<tr>
<td>Plethysmography</td>
<td>The study of the variations in the volume of an organ or limb resulting from changes in the amount of fluid present or passing through it.</td>
</tr>
<tr>
<td>Polarization</td>
<td>The disturbance of the electric field induced by the charge distribution in a region.</td>
</tr>
<tr>
<td>Protoplasm</td>
<td>The substance inside the plasma membrane of the cell. It is composed by the cytoplasm and the nucleus</td>
</tr>
<tr>
<td>Relaxation</td>
<td>The return process of a system to equilibrium after a disturbance</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>The restoration of blood flow to an area or part that was previously ischemic</td>
</tr>
<tr>
<td>Resistance</td>
<td>It is a measure the opposition of the material to the flow of electric charges and it is measured in ohms, ( \Omega ). It is the reciprocal of the conductance.</td>
</tr>
<tr>
<td>Resistivity</td>
<td>It is a measure of the ability of a material to oppose the flow of electrical current and it is measured in Ohm meter, ( \Omega ) m. It is the reciprocal of the conductivity.</td>
</tr>
<tr>
<td>Stroke</td>
<td>A type of cerebrovascular disease caused by an impairment of blood flow or oxygenation of Central Nervous System tissue, resulting in injured CNS tissue</td>
</tr>
<tr>
<td>Viscosity</td>
<td>The internal resistance to flow in a fluid due to intermolecular friction. It is inversely proportional to temperature</td>
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Part II
BRAIN ELECTRICAL IMPEDANCE AT VARIOUS FREQUENCIES: THE EFFECT OF HYPOXIA

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Brain Electrical Impedance at various Frequencies: the Effect of Hypoxia

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Abstract: Non-invasive multi-frequency measurements of transcephalic impedance, both reactance and resistance, can efficiently detect cell swelling of brain tissue and can be used for early detection of threatening brain damage. We have performed experiments on piglets to monitor transencephalic impedance during hypoxia. The obtained results have confirmed the hypothesis that changes in the size of cells modify the tissue impedance. During tissue inflammation after induced hypoxia, cerebral tissue exhibits changes in both reactance and resistance. Those changes are remarkably high, up to 71% over the baseline, and easy to measure especially at certain frequencies. A better understanding of the electrical behaviour of cerebral tissue during cell swelling would lead us to develop effective non-invasive clinical tools and methods for early diagnosis of cerebral edema and brain damage prevention.

Keywords— Bioimpedance, Brain Damage, Cell Swelling, Cerebral Edema, Hypoxia, Ischemia, Tissue inflammation.

1. INTRODUCTION

Society’s costs for neuropsychiatric diseases are by far the largest compared to all disease groups. The total cost for medical care of brain science-related disorders in E.U, US and Japan is estimated to 400 BUSD (Swedish Brain Power 2001). Many nervous system related disorders are cause by hypoxic brain damage as a result of severe oxygen lack and/or cerebral blood circulation failure (hypoxia/ischemia) during intensive care, surgery or labour. For instance one of 500 newborns suffers severe perinatal asphyxia (Lingwood et al. 2003) and up to 48% of all patients suffer from cognitive dysfunction after cardiac surgery (Toner et al. 1998). Currently there are no efficient non-invasive techniques to detect clinical situations of impending hypoxic brain damage.

Hypoxia/Ischemia, an important and common cause of cell injury, impinges on the aerobic oxidative respiration of the cell (Cotran et al. 1989). Lack of oxygen in the
cell reduces the cellular energy production thus making the cell membrane lose some of its transport and regulation functions. Without these membrane functions the intracellular concentration of solute, ions and catabolites, increases. An increase accompanied by an isosmotic increment of water and resulting in cell swelling, also denominated cellular edema (Klatzo 1994).

Cellular edema implies a redistribution of intracellular and extracellular fluids followed by a change in the tissue structure. Biological tissue consists of an aggregation of cells in a conductive fluid (Roth 2000), and the electrical impedance of tissue depends on the cellular structure and composition (Cole and Cole 1941), see Figure 1. Cell swelling causes structural changes in the tissue, resulting in changes in the electrical impedance of the tissue (Cole and Curtis 1950), see (1).

\[
Z = \frac{(1 - f)r_e + (2 + f)\left( r_i - \frac{j}{\omega c_m a} \right)}{(1 + 2f)r_e + 2(1 - f)\left( r_i - \frac{j}{\omega c_m a} \right)}
\]  

Where:

- \(Z\) = specific impedance of the tissue, \(\Omega \cdot \text{cm}\)
- \(r_e\) = resistivity of extracellular fluid, \(\Omega \cdot \text{cm}\)
- \(r_i\) = resistivity of cytoplasm, \(\Omega \cdot \text{cm}\)
- \(c_m\) = surface membrane capacity, Farads/cm²
- \(a\) = cell radius, cm
- \(f\) = volume factor of cells concentration
- \(\omega\) = angular frequency, rad/s
- \(j\) = the imaginary unit \(\sqrt{-1}\)

The potential application of electrical bioimpedance measurement for tissue identification and/or monitoring of structural changes have been extensively studied by many authors during the last 15 years (Lingwood et al. 2002a; Lingwood et al. 2002b; Lee et al. 2003), especially in the field of Electrical Impedance Tomography (EIT) (Holder 1992b, a; Vauhkonen et al. 1999; Tidswell et al. 2001; Yerworth et al. 2003).

The few studies that focus on hypoxic brain damage detection have confirmed the association between hypoxic episodes and changes of impedance in the brain (Holder 1992a; Lingwood et al. 2002b; Lingwood et al. 2003). The electrical impedance consists of two components: resistance and reactance, but most of the research to date has been focused on the resistance. In most of the previous studies, the impedance of the tissue has been measured at low frequencies, 50 kHz. However reactance and resistance of biological tissue exhibit a different behavior regarding frequency (Cole 1928, 1932). Therefore at certain frequencies reactance monitoring may represent better changes in fluid distribution on the tissue structure.

In order to detect and prevent brain cellular edema and potential brain damage, there is a need for a methodology that allows the monitoring of signs of threatening conditions before irreversible changes have taken place. Currently, bioelectrical impedance measurement instrumentation is used at hospitals for different clinical
purposes, e.g. cardiac-respiratory measurements and water body contents. It is inexpensive, and does not represent risk or discomfort for the patient. It can be applied non-invasively, and as it is portable it can be use at the bedside.

The aim of this study is to investigate the relation between resistance and reactance in transencephalic impedance at frequencies in the range 20 kHz to 750 kHz and the dynamics, time relation, during acutely induce hypoxia.

2. METHODOLOGY

An experiment was designed to invasively measure brain impedance on piglets before, during and after hypoxia. Continuous transcephalic impedance measurements are used as indicators of change in fluid volumes in brain tissue. The performed study was approved by the Ethics Committee for Animal Research of Göteborg University.

Animal Preparation and Induced Hypoxia

Newborn pigs, 1-4 days old, were anaesthetized with ketamine/chloralose and ventilated to maintain normal blood gases. EEG and transencephalic impedance were recorded together with arterial blood pressure and heart rate. After a one hour long control period, 45 min of severe hypoxia was institute by decreasing oxygen in the inhaled gas mixture to 6 per cent. EEG responded by a rapid loss of activity and an isoelectric EEG was maintained for 45 minutes, then oxygen was added to the gas mixture and normal oxygenation was maintained for the following 16 hours.

The subjects were sacrificed immediately at the end of the experiments by a lethal overdose of pentothal.

Measurement of Transecephalic Impedance

Four burr holes were drilled through the scalp in the positions P3, P4, C3 and C4. Silver rod electrodes of 2,5mm were screwed into the holes with the surface resting on the dura. Electrical bioimpedance was measured using a custom-made impedance meter (Jakobsson 2000), based on the 4-electrode method. A sine wave alternating current of 1 mA peak-to-peak was applied for continuous electric stimulation through electrodes C3-C4. Resistance and reactance were measured from electrodes P3-P4 in the frequency range from 20 kHz to 750 kHz.
Brain Electrical Impedance at various Frequencies: the Effect of Hypoxia

Data measurements were processed and stored with a customized SACS® application (Lindecrantz et al. 1999) for physiological monitoring instrumentation.

3. RESULTS

Performed measurements showed that the effective electrical impedance of the brain changes abruptly during and after hypoxia. In Figure 2, the evolution of the cerebral resistance at 50 kHz is shown. The magnitude of the resistance remained practically invariable before hypoxia was induced. Resistance began to increase upon induced hypoxia. In 45 minutes of hypoxia the resistance increased from 52 Ohms to 85 Ohms. After subject resuscitation, the resistance kept increasing, for a short period of time, reaching a maximum value of 89 Ohms; 71 % increment over the pre-hypoxic value. In some subjects after resuscitation, the resistance decreased towards baseline for the remainder of the experiment. In other subjects the resistance decreased approximately for one hour before it increased again.

It was observed that the augmentation ratio (proportional increment of magnitude regarding the initial value given in %) of the resistance and reactance exhibited certain frequency dependency, see table I and Figure 3. A measured increment in resistance of 24.3% at 20 kHz, was 22.1% at 200 kHz and 11.1% at 500 kHz, while at 750 kHz, the resistance decreased 5.2%. Reactance exhibited an augmentation ratio value centered approximately around 30% over the complete measured frequency range.

![Figure 2. Evolution of transencephalic resistance during induced hypoxia measured at 50 kHz. Resistance in Ohms in the left axis and difference respect baseline in the right axis.](image)

**Table I.** Augmentation Ratio for Reactance and Resistance at various Frequencies

<table>
<thead>
<tr>
<th>Frequency, in kHz</th>
<th>Reactance (Δvalue)/Initial value) x 100</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>23.9 %</td>
<td>24.3 %</td>
</tr>
<tr>
<td>100</td>
<td>24.0 %</td>
<td>21.5 %</td>
</tr>
<tr>
<td>200</td>
<td>30.9 %</td>
<td>22.8 %</td>
</tr>
<tr>
<td>300</td>
<td>29.7 %</td>
<td>24.6 %</td>
</tr>
<tr>
<td>500</td>
<td>36.6 %</td>
<td>11.1 %</td>
</tr>
<tr>
<td>750</td>
<td>33.6 %</td>
<td>-5.2 %</td>
</tr>
</tbody>
</table>

Figure 3 shows the time evolution of cerebral impedance using complex impedance plots. It contains three impedance plots measured at one hour before hypoxia was induced, one hour and 30 minutes, and two hours and 30 minutes after
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hypoxia. We observed that the radius and the center of the impedance plot changed and shifted, respectively, during hypoxia.

**Figure 3.** Complex impedance plot for the cerebral impedance at various time intervals during hypoxia. Frequency range from 20 kHz to 750 kHz.

4. DISCUSSION

Our study confirms that deprivation of oxygen in newborn piglets produces changes in brain impedance. Changes in impedance are expected during hypoxia considering the structural changes that cellular edema introduces in tissue. This behavior of the effective electrical bioimpedance can be deduced from Figure 1 and (1).

Immediately after induced hypoxia, the cerebral resistance increases abruptly at low frequencies. This behavior is consistent with results reported by Holder in (Holder 1992a) and Lingwood in (Lingwood et al. 2002b; Lingwood et al. 2003). This increase of resistance is intrinsic to the formation of cellular edema; the cells swell, increasing the intracellular space and decreasing the extracellular space. The resistance increment is noticed especially at low frequencies because the electrical current flows essentially through the extracellular space at low frequencies (Roth 2000).

The augmentation ratio of the resistance showed a decreasing trend regarding frequency. This trend is related to the capacitive effect of the cellular membrane. This decreasing trend results in the augmentation ratio leading to negative values at high frequencies. It should be noted that it was an unexpected result and since there are no previous reported experiments about this specific phenomenon to support or disregard this finding, we think it must be subject to further study.

The measurements have been performed invasively, therefore most of the applied current was used in the measurements and the changes in impedance were measured and detected very clearly. Non-invasive impedance measurements have to deal with the effect of the skull and the scalp, thus we expect the changes in impedance will not be as large as measured here, but the changes will still easily perceptible as it has been reported by Lingwood in (Lingwood et al. 2002b) and Holder in (Holder 1992a).
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The relation between frequency and impedance, both real and imaginary components, gives a clear indicator of suitable frequency ranges for detection of cell swelling in the brain.

5. CONCLUSION

The impedance signal displays a rapid response to hypoxia. The response appears to be graded: the degree of impedance change reflects the severity of the insult to the brain. Non-invasive multi-frequency measurement of brain electrical complex impedance may become a valuable method for early detection of brain cellular edema.

The frequency dependency of the resistive and reactive parts of the impedance confirms the electrical equivalent of the brain tissue and supports the hypothesis that cell swelling is the major mechanism behind the changes in impedance.

Measurements of resistance and reactance at various frequencies may be the foundation on which to develop new monitoring tools for early diagnosis of brain cellular edema. A clinical method of cerebral monitoring based on our results will require further studies of the behavior of the impedance.

There are some uncertainties about the behavior of the effective complex impedance of the biological tissue during cell swelling. These uncertainties should be investigated in order to obtain a better understanding of electrical phenomena on biological tissue under structural changes. The feasibility to the use of non-invasive electrodes must also be addressed.

REFERENCES

(Holder D S 1992a). Detection of cerebral ischaemia in the anaesthetized rat by impedance measurement with scalp electrodes: implications for non-invasive imaging of stroke by electrical impedance tomography Clinical Physics and Physiological Measurement (1): 63-75
(Holder D S 1992b). Detection of cortical spreading depression in the anaesthetized rat by impedance measurement with scalp electrodes: implications for non-invasive imaging of the brain with electrical impedance tomography Clinical Physics and Physiological Measurement (1): 77-86
(Jakobsson U 2000). Investigations of the characteristic impedance of the brain and development of an impedance meter for a wide frequency spectrum Chalmers Univ. of Technology
(Klatzo I 1994). Evolution of brain edema concepts Acta Neurochir Suppl 60 3-6
Brain Electrical Impedance at various Frequencies: the Effect of Hypoxia


Bioelectrical Impedance during Hypoxic Cell Swelling: Modelling of Tissue as a Suspension of Cells

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Abstract: Non-invasive multi-frequency measurements of transcephalic impedance, both reactance and resistance, can efficiently detect cytotoxic edema in brain tissue and can be used for early detection of threatening brain damage. The model of biological tissue as a suspension of cells can be used as a valuable guide to identify the optimum range of frequencies for electrical impedance monitoring to detect cell swelling efficiently. We have performed experiments on piglets to monitor transencephalic impedance during hypoxia. The obtained results have confirmed the hypothesis that changes in the size of cells modify the tissue impedance. During tissue inflammation after induced hypoxia, cerebral tissue exhibits changes in both reactance and resistance. Those changes in impedance exhibit certain dependency of frequency in concordance with the suspension of cells model. The experimentally observed changes are remarkably high, up to 71% over the baseline, and easy to measure especially at certain frequencies. A better understanding of the electrical behaviour of cerebral tissue during cell swelling may lead the development effective non-invasive clinical tools and methods for early diagnosis of cerebral edema and brain damage prevention.

Keywords: Bioimpedance, Brain Damage, Cell Swelling, Cerebral Edema, Cole Plot, Hypoxia, Ischemia, Suspension of Cells, Tissue inflammation.

1. INTRODUCTION

Society’s costs for neuropsychiatric diseases are by far the largest compared to all disease groups. The total cost for medical care of brain science-related disorders in EU, US and Japan is estimated to 400 BUSD (Swedish Brain Power 2001). Many disorders related to the nervous system are caused by hypoxic brain damage as a result of severe oxygen lack and/or cerebral circulation failure (hypoxia/ischemia) during intensive care, surgery or labour. For instance one out of 500 newborns suffers severe perinatal asphyxia (Lingwood et al. 2003) and up to 48% of all patients suffer from cognitive dysfunction after cardiac surgery (Toner et al. 1998). Currently there are no...
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Fig. 1. (a) Suspension of spherical cells. (b) An equivalent electric circuit of a suspension of spherical cells.

Efficient non-invasive techniques to detect clinical situations of impending hypoxic brain damage.

Hypoxia/ischemia, an important and common cause of cell injury, impinges on the aerobic oxidative respiration of the cell (Cotran et al. 1989). Lack of oxygen in the cell reduces the cellular energy production thus causing the cell membrane to lose some of its transport and regulation functions. Without these membrane functions the intracellular concentration of solute, ions and catabolites increases, an increase accompanied by an isosmotic increment of water and resulting in cell swelling, denominated cellular or cytotoxic edema (Klatzo 1994).

Cellular edema implies a redistribution of intracellular and extracellular fluids followed by a change in the tissue structure. Biological tissue consists of an aggregation of cells in a conductive fluid (Roth 2000), and the electrical impedance of tissue depends on the cellular structure and composition (Cole and Cole 1941). Cell swelling modifies the size of the cell, thus it causes structural changes in the tissue, resulting in changes in the electrical impedance of the tissue (Cole and Curtis 1950).

The model of biological tissue as a suspension of cells, Figure 1a, has been used for the last 80 years (Fricke and Morse 1925; Cole 1928; Schwan 1957) to approximately describe the frequency dependency of the electrical impedance, see equation (1). The equivalent circuit of a suspension of spherical cells is shown in Figure 1b, where \( R_e \) represents the resistance of the interstitial medium, \( R_i \) of the intracellular volume and \( C_m \) the capacitance of surface of the cellular membranes (Cole 1928).

Equation (1)

\[
Z = r_e \frac{(1 - f)r_e + (2 + f)\left(r_i + \frac{r_m}{a} - \frac{i}{\omega c_m a}\right)}{(1 + 2f)r_e + 2(1 - f)\left(r_i + \frac{r_m}{a} - \frac{i}{\omega c_m a}\right)}
\]

Where in 2D:

\[
N_c = \text{number of Cells} \quad \Rightarrow f = \frac{N_c \times \pi \times a^2}{S_t}
\]

Where:

\( Z = \) specific impedance of tissue, \( \Omega \times \text{cm} \)

\( r_e = \) resistivity of extracellular fluid, \( \Omega \times \text{cm} \)

\( r_i = \) resistivity of cytoplasm, \( \Omega \times \text{cm} \)

\( c_m = \) surface membrane capacity, Farads/cm²

\( a = \) cell radius, cm

\( f = \) volume factor of concentration of cells

\( \omega = \) angular frequency, radians×s⁻¹

\( i = \) the imaginary unity operator \( \sqrt{-1} \)
The aim of this study is to investigate the relation between electrical resistance and reactance in biological tissue over a wide range of frequencies and tissue structural changes, during cell swelling.

2. METHODOLOGY

Equation (1) was developed and used to calculate the effective behavior of the electrical bioimpedance of tissue regarding frequency and tissue structure. The obtained theoretical behavior was compared with the results of an experiment designed to invasively measure brain impedance on piglets before, during and after hypoxia (Seoane et al. 2004b). The performed animal experiment was approved by the Ethics Committee for Animal Research of Göteborg University.

Suspension of Cells Model

A two-dimensional approach to biological tissue modeled as a suspension of spherical cells on conducting fluid was used (Roth 2000). The mathematical representation of the model, Equation (1), was developed to calculate the behavior of the complex impedance regarding frequency, size of cells and volume factor of concentration of cells. Both, real and imaginary components were extracted and represented using complex impedance plots for different values of cell radius. The modeled tissue contained $10^8$ cells and has size of $1\text{cm}^2$. The surface membrane resistivity of the cells was neglected, $r_m = 0$.

Animal Preparation and Induced Hypoxia

Newborn pigs, 1-4 days old, were anaesthetized with ketamine/chloralose and ventilated to maintain normal blood gases. EEG and transencephalic impedance were recorded together with arterial blood pressure and heart rate. After a one hour long control period, 45 min of severe hypoxia was instituted by decreasing oxygen in the inhaled gas mixture to 6 per cent. EEG responded by a rapid loss of activity and an isoelectric EEG was maintained for 45 min. Then oxygen was added to the gas mixture and normal oxygenation was maintained for the following 16 hours. The subjects were sacrificed immediately at the end of the experiments by a lethal overdose of pentothal.

Measurement of Transcephalic Impedance

Four burr holes were drilled through the scalp in the positions P3, P4, C3 and C4. Silver rod electrodes of 2,5mm were screwed into the holes with the surface resting on the dura. Electrical bioimpedance was measured using a custom-made impedance meter (Jakobsson 2000), based on the four–electrode method. A sine wave alternating current of 1 mA peak-to-peak was applied for continuous electric stimulation through electrodes C3-C4. Resistance and reactance were measured from electrodes P3-P4 in the frequency range from 20 kHz to 750 kHz. Data measurements were processed and stored with a customized (Lindecrantz et al. 1999)] SACS® application for physiological monitoring instrumentation.
3. RESULTS

The obtained results, theoretical in Figures 3 and 5 and experimental in Figure 4 and 6, prove that the complex impedance show signs of an evident dependency of the cells size. Both impedance plots, Figures 3 and 4, show a analogous behavior, the radius of the impedance plot increases with the size of the cells; an increase followed by a shift of the center of the impedance plot over the resistance axis. The behavior of the effective resistance of the model, regarding the size of the cells, over frequency also fits the measured data, see Figures 5 and 6. Notice that, in Figures 3 and 5, the volume factor of cell concentration, \( f \), is 0.75, 0.80 and 0.85 for radius values, \( a \), equal to 5.0, 5.17 and 5.33 \( \mu \text{m} \) respectively.

4. CONCLUSION

From the results we can easily observe that theoretical and experimental results fit well. The theoretical results from the suspension of cells model also support the findings previously reported in (Seoane et al. 2004b) about the measured decrease of
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total resistance during hypoxia at around 750 kHz while it increased at lower frequencies.

To consider biological tissue as a suspension of spherical cells in a conducting fluid and to calculate the effective impedance of the model using a two-dimensional approach has some inherent limitations. Nevertheless the obtained results indicate that the suspension of cells model provides us with valuable information about the behavior of electrical bioimpedance of tissue regarding cells size and volume concentration over frequency. Simulations based in the suspension of cells model can be used to obtain an estimation of the behavior of resistance and reactance of biological tissue regarding frequency and cells size, which may be useful in the development of efficient measurement instrumentation for monitoring of tissue inflammation.

Computational electrical analysis based in numerical field calculation, simulations based in the Finite Element Method or combinations of both would probably obtain more accurate results. Ramos in (Ramos et al. 2003) calculated the electrical characteristics skeletal muscle tissue using numerical field calculations and Pavlin in (Pavlin et al. 2002) calculated the conductivity of a three-dimensional suspensions of cells using the Finite Element Method, but no results about the behavior of the effective bio-impedance regarding size of cells were reported.

REFERENCES

(Fricke H and Morse S 1925). The Electric Resistance and Capacity of Blood for Frequencies Between 800 and 4.5 Million Cycles Journal of General Physiology 9 153-67
(Jakobsson U 2000). Investigations of the characteristic impedance of the brain and development of an impedance meter for a wide frequency spectrum Chalmers Univ. of Technology
(Klatzo I 1994). Evolution of brain edema concepts Acta Neurochir Suppl 60 3-6
(Pavlin M, Slivnik T and Miklavcic D 2002). Effective conductivity of cell suspensions Biomedical Engineering, IEEE Transactions on 49 (1): 77-80
Bioelectrical Impedance during Hypoxic Cell Swelling: Modelling of Tissue as a Suspension of Cells


(Schwan H P 1957). Electrical properties of tissue and cell suspensions Adv Biol Med Phys 5 147-209


EVOLUTION OF CEREBRAL BIOELECTRICAL RESISTANCE AT VARIOUS FREQUENCIES DURING HYPOXIA IN FETAL SHEEP

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Evolution of Cerebral Bioelectrical Resistance at various Frequencies during Hypoxia in Fetal Sheep

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1. INTRODUCTION

Perinatal asphyxia is a significant cause of mortality, neuro-developmental disability and long-term neurological morbidity in term newborn infants. Neural rescue therapies have been tested in animal with satisfactory results (Gunn et al. 1998) and clinical trials on human population are in progress. A quick and reliable detection of the hypoxia would allow an earlier initiation of the rescue therapy. Cerebral electrical bioimpedance can detect cytotoxic edema caused by hypoxic cell swelling (Williams et al. 1991).

2. METHODS

A group (n = 4) of fetal sheep at 93-96 days of gestation was subjected to 25 minutes of induced hypoxia by occlusion of the umbilical cord with a vascular occluder. Through surgically implanted electrodes, brain electrical bioimpedance (only the real part, the resistance, not the reactance) was measured at 30 kHz and 200 kHz using a 4-electrode method custom-made impedance meter.

3. RESULTS

In Figure 1 we can see the changes in the bioelectrical resistance of the brain measured at two different frequencies during induced hypoxia. Initially there is a stable baseline. At onset of hypoxia there is a period of latency, followed by a phase of increasing resistance at both frequencies. Resistance keeps increasing throughout the hypoxic period and even a while after reoxygenation. Resistance measured at 30 kHz shows quicker and more noticeable changes than measured at 200 kHz. See table 1).

Table 1. Mean values of the evolution of brain electrical resistance in fetal sheep during induced hypoxia at 30 kHz and 200 kHz.

<table>
<thead>
<tr>
<th>kHz</th>
<th>Mean latency before noticeable changes (mm:ss)</th>
<th>Mean time to 10% over baseline (mm:ss)</th>
<th>Mean peak (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>07:47</td>
<td>16:37</td>
<td>35</td>
</tr>
<tr>
<td>200</td>
<td>08:38</td>
<td>17:51</td>
<td>30</td>
</tr>
</tbody>
</table>
Evolution of Cerebral Bioelectrical Resistance at various Frequencies during Hypoxia in Fetal Sheep

Figure 1. Evolution of transencephalic electrical resistance in a fetal sheep during induced hypoxia, at 30 kHz and 200 kHz.

4. DISCUSSION & CONCLUSIONS

Resistance measured at 30 kHz starts to increase earlier and increases more, proportionally and in absolute terms, than resistance measured at 200 kHz. These observations confirm that measurements at low frequencies are a better indicator of cell swelling, in time and magnitude, than measurements at higher frequencies.

Although bioelectrical resistance indicates fairly well the evolution of cell swelling, the results show that measurements of resistance at a single-frequency have certain limitations in early detection of hypoxia. For this purpose multi-frequency measurements of complex bioimpedance, resistance and reactance, may be a quicker and better indicator (Seoane et al. 2004a).

REFERENCES:


(Williams C E, Gunn A and Gluckman P D 1991). Time course of intracellular edema and epileptiform activity following prenatal cerebral ischemia in sheep Stroke 22 (4): 516-21
SPECTROSCOPY STUDY OF THE DYNAMICS OF THE TRANSENCEPHALIC ELECTRICAL IMPEDANCE IN THE PERINATAL BRAIN DURING HYPOXIA.

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Spectroscopy Study of the Dynamics of the Transencephalic Electrical Impedance in the Perinatal Brain during Hypoxia

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Abstract: Hypoxia/ischemia is the most common cause of brain damage in neonates. Thousands of newborn children suffer from perinatal asphyxia every year. The cells go through a response mechanism, during hypoxia/ischemia, to maintain the cellular viability and, as a response to the hypoxic/ischemic insult, the composition and the structure of the cellular environment is altered. The alterations in the ionic concentration of the intra- and extracellular and the consequent citotoxic edema, cell swelling, modify the electrical properties of the constituted tissue. The changes produced can be easily measured using electrical impedance instrumentation. In this manuscript we report the results from an impedance spectroscopy study on the effects of the hypoxia on the perinatal brain. The transencephalic impedance, both resistance and reactance, was measured in newborn piglets using the 4-electrode method in the frequency range from 20 kHz to 750 kHz and the experimental results were compared with numerical results from a simulation of a suspension of cells during cell swelling. The experimental results make clear the frequency dependency of the bioelectrical impedance, confirm that the variation of resistance is more sensitive at low than at high frequencies and show that the reactance changes substantially during hypoxia. The resemblance between the experimental and numerical results proves the validity of modelling tissue as a suspension of cells and confirms the importance of the cellular edema process in the alterations of the electrical properties of biological tissue. The study of the effects of hypoxia/ischemia in the bioelectrical properties of tissue may lead to the development of useful clinical tools based on the application of bioelectrical impedance technology.

1. INTRODUCTION

Hypoxia/Ischemia is responsible for many disorders, disabilities and deaths related to the nervous system as a result of severe oxygen lack and/or cerebral circulation failure. For instance, up to 48% of all patients suffer from cognitive dysfunction after cardiac surgery with imposed cardiac arrest (Toner et al. 1998) and up to 1.5% of newborns suffer from perinatal asphyxia (Legido et al. 2000). This high incidence rate of hypoxic/ischemic brain damage not only influences dramatically the life of thousands of affected people and their families, it also entail a huge economic burden for society, billions of euros every year worldwide.
Spectroscopy Study of the Dynamics of the Transencephalic Electrical Impedance in the Perinatal Brain during Hypoxia

Perinatal asphyxia is one of the diseases related to hypoxic/ischemic brain damage and it is the most significant cause of mortality, neuro-developmental disability and long-term neurological morbidity in term newborn infants. Neural rescue therapies have been tested on animal with satisfactory results (Gunn et al. 1998) and clinical trials of hypothermia therapy are in progress on human population. Even when a successful therapy may allow the recovery of the affected patients as a requirement prior to therapy initiation, the hypoxic situation needs to be clearly and efficiently detected. Moreover, the time window between insult and start of therapy appears to be narrow (Vannucci and Perlman 1997; Roelfsema et al. 2004).

1.1. Hypoxia and cellular edema

Hypoxia/Ischemia is an important and common cause of cell injury. It affects the cellular metabolism, impinging on the aerobic oxidative respiration of the cell (Cotran et al. 1989). Lack of oxygen in the cell forces the cell to resort to anaerobic metabolism with glycogen-glucose breakdown halted at the lactate level. The reduced capability of the cell to produce energy forces the cell membrane to lose some of its regulation and active transport functions. This leads to a rapid accumulation of osmotic active products in the intracellular space, an accumulation accompanied by an isosmotic increment in water and resulting in an inevitable intracellular swelling. This intracellular swelling is denominated cellular or cytotoxic edema (Klatzo 1994) and it characterises the early phase of hypoxia/ischemia.

1.2. Electrical Bioimpedance

Biological tissue is a dielectric conductive material and electrical current passes through the tissue when a difference of electric potential is applied on the tissue in a closed electric circuit. Biological tissue is often considered as an aggregation of cells, with a semi-permeable membrane containing the intracellular fluid, surrounded by the extracellular fluid. Both intracellular and extracellular fluids are conductive, therefore exhibit certain resistance, and the cellular membrane acts as a capacitor, Figure 1.

1.3. Effects of cell swelling on the bioelectrical impedance of the tissue

Cell swelling causes cellular edema, which implies a redistribution of fluids between the intracellular and extracellular spaces. This fluid redistribution causes changes in the structure of the tissue, resulting in changes in the electrical

Figure 1. A) and B) show the pathways of the electrical current through biological tissue; A) low frequency, B) high frequency. C) shows the electrical circuit model equivalent of biological tissue; three elements 2R-1C parallel type.
bioimpedance (Van Harreveld 1957). The electrical impedance consists of two components: resistance and reactance, and both depend on the shape of the cells, tissue structure and tissue composition. Therefore our working hypothesis is that during cell swelling both of them may be modified notably and in theory substantial changes are expected, especially in the reactive part.

Previous experimental studies focused on hypoxic/ischemic brain damage have successfully confirmed the association between hypoxic/ischemic events and changes of electrical bioimpedance in the brain (Van Harreveld 1957; Williams et al. 1991; Holder 1992; Lingwood et al. 2002). In this study, under our working hypothesis, we take the step of studying the dynamics of the impedance during normoxia followed by an induced hypoxic event and further during a phase of reoxygenation. We study both resistance and reactance and their dependency on frequency; the variations are interpreted in relation to the model of biological tissue as a suspension of spherical cells, Figure 2 and equation (1) from Cole (Cole 1928).

2. METHODOLOGY

A bioimpedance spectroscopy study of biological tissue during cell swelling has been performed. An experiment with live animals was performed to invasively measure transencephalic electrical impedance on piglets before, during and after hypoxia. Numerical calculations based on the model of tissue as a suspension of cells were performed to calculate the effective behaviour of the electrical bioimpedance of tissue regarding the frequency, tissue structure, and the radius of the cells.

The performed animal experimentation was approved by the Ethics Committee for Animal Research of Göteborg University.
2.1. Animal Preparation and Induced Hypoxia

Newborn pigs, 1-4 days old, were anaesthetised with ketamine for induction and chloral hydrate for maintenance and then ventilated to maintain normal blood gases. Transencephalic impedance was recorded together with arterial blood pressure, heart rate and Electroencephalic activity (EEG). After a one-hour-long control period, 45 min of severe hypoxia was instituted by decreasing oxygen in the inhaled gas mixture to 6% to induce loss of EEG activity. Then oxygen was added to the gas mixture and normal oxygenation was maintained for the following 16 hours. The subjects were sacrificed immediately at the end of the experiments by a lethal overdose of pentothal.

2.2. Measurement of Transencephalic Impedance

Four burr holes were drilled through the scalp in positions P3, P4, C3 and C4. See Figure 3. Silver rod electrodes of 2.5 mm of diameter with rough surface were screwed into the holes with the surface resting on the dura. Electrical bioimpedance was measured using a frequency selectable custom-made impedance meter (Jakobsson 2000), based on the 4-electrode current-injection/voltage-measurement method (Ackmann et al. 1984).

A symmetric sinusoidal current of 500 µA of peak value was applied for continuous electric stimulation. The current was injected through the current electrodes placed on C3 and C4. Complex electrical bioimpedance, resistance and reactance, was measured from potential-sensing electrodes placed on P3 and P4.

The dynamics of the resistance was studied in seven (7) subjects at the frequencies 50 kHz and 200 kHz. A more elaborate study was performed in two (2) subject where the measurements were made in the frequency range from 20 to 750 kHz. In these two (2) subjects the impedance, resistance and reactance, was measured and the results were compared with the suspension of cells model.

![Figure 3](image_url). The four-electrode method and the placement of electrodes following the standard 10-20 system on the head of a piglet. 2 electrodes inject the current while the resulting voltage through the tissue is measured with the other two. Current injecting electrodes placed on C3 and C4 and potential sensing electrodes placed on P3 and P4.
2.3. Numeric Calculations on the Suspension of Cells Model

The impedivity of a suspension of spherical cells was calculated using the expression derived by Cole (Cole 1928) applying the articles 313 and 314 of Maxwell’s Treatise (Maxwell 1873). The impedivity obtained was used to calculate the impedance of a cylindrical conductor. The limits and accuracy in calculation of the electrical bioimpedance of tissue modeled as a suspension of cells have been studied and reported by Cole (Cole et al. 1969; Cole 1976).

Equation (1) was fitted to geometrically satisfied three-dimensions using equation (2) to define the volume factor, $f$, with respect the radius of the cells $a$. Both, real and imaginary components were calculated with equation (1) and the resulting values were represented using complex impedance plots for different values of cell radii. The model represented a cylindrical portion of tissue of radius 20 mm and length 20 mm containing $10^9$ cells. The plasma membrane was considered an ideal capacitor. Thus $\zeta_m$ in equation (1) is substituted by $(j\omega c_m)^{-1}$. Where $j$ is the imaginary operator $\sqrt{-1}$, $\omega$ is the angular frequency in radians$\times$s$^{-1}$ and $c_m$ is the surface membrane capacity, in Farads$\times$cm$^{-2}$. The resistivity values used in the numerical calculation are electrical properties of myelinated nerve fibers extracted from (Malmivuo and Plonsey 1995).

\begin{equation}
\Delta f = \frac{N_c \times 4 \times \pi \times a^3}{3 \times V_t}
\end{equation}

3. RESULTS

The measurements performed showed that the electrical bioimpedance of the brain changes notably during and after hypoxia with respect to the normoxic baseline value.

3.1. Dynamics of resistance – normoxic phase

During normoxia, before the hypoxic insult was induced, the subjects, (n=7), presented a stable brain electrical resistance baseline, for each subject, though, at significantly different values. The average resistance measured at 50 kHz was 55.4 $\Omega$ with 16.4 $\Omega$ of a standard deviation (SD) and 47.6 $\Omega$ with 16.5 $\Omega$ of SD measured at 200 kHz. Most of the subjects presented baseline values close to the average value, but one subject showed a very high baseline value, 83.9 $\Omega$ at 50 kHz and 73.9 at 200 kHz, and another subject showed a noticeable low baseline, 32.2 $\Omega$ at 50 kHz and 20 at 200 kHz. See table I.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
$f$(kHz) & $\text{max (}\Omega\text{)}$ & $\text{min (}\Omega\text{)}$ & $\text{mean (}\Omega\text{)}$ & $\text{SD (}\Omega\text{)}$ \\
\hline
50   & 83.9 & 32.3 & 55.3 & 16.4 \\
200  & 73.9 & 20 & 47.6 & 16.5 \\
\hline
\end{tabular}
\caption{Statistics of the measured resistance baseline previous to hypoxia. Measurements performed at 50 kHz and 200 kHz over a set of 7 subjects.}
\end{table}
3.2. Dynamics of resistance – hypoxic phase

During hypoxia, the transencephalic resistance increased remarkably over the established baseline. The same individual specificity observed for the value of the baseline was observed for the time evolution of the resistance measured during hypoxia.

Figure 4 shows three examples of the evolution of resistance. The resistance tends to increase in every subject but with different slopes reaching different maximum values. Resistance began to increase right after hypoxia was initiated. It was observed that during 45 minutes of hypoxia, the resistance at 50 kHz increased by a minimum of 23% and by a maximum of 119% over the pre-hypoxic value. After the normal oxygenation was re-instituted, the resistance kept increasing for a short period of time, after which the resistance reached the maximum value; at least a 25% increment in some cases and up to a 121% over the normoxic value.

The evolution of the resistance measured at 200 kHz was identical to the evolution of the resistance at 50 kHz for every piglet. The only difference was that the values of the magnitude and the changes were smaller.

![Figure 4](image)

**Figure 4.** Time evolution of the cerebral electrical resistance in the asphyxiated piglet measured at 50 kHz in three different subjects. 45 minutes of hypoxic period marked by the shadowed region.

3.3. Dynamics of resistance – post-hypoxic phase

At low frequencies, during the post-hypoxic phase three different dynamics were observed in the resistance. In the first group of piglets, the resistance decreased towards baseline and after the baseline value was restored, the resistance value stayed stable for the remaining part of the experiment. In a second group, the resistance after reaching the baseline value and keeping stable for a certain period, started to increase again. In the third group, after a short period decreasing, the resistance started to increase again, before the baseline value was reached.

3.4. Complex Electrical Bioimpedance during Hypoxia

In both piglets, the complex bioimpedance, both reactance and resistance, across the brain tissue exhibited changes during hypoxia and after hypoxia was instituted. Figure 5 shows the time evolution of the cerebral electrical impedance in one of the subjects before, during and after hypoxia. The behaviour of the cerebral impedance in the other piglets followed a similar evolution. The plots contained in
Figure 5. Plot of transencephalic electrical bioimpedance on the brain of a piglet at three different moments during hypoxic cell swelling. Frequency range from 20 kHz to 750 kHz.

Figure 6 are complex impedance plots, illustrating absolute reactance value versus resistance over the frequency range from 20 kHz to 750kHz.

We observed that during hypoxia the radius of the impedance plot increased and the centre was shifted to higher values of resistance. After oxygenation was re-instituted, the radius of the plot decreased and the centre shifted back towards the normoxic value.

The numerical results obtained from the calculations based on the suspension of cells and the experimentally measured values matched well. Both sets of bioimpedance plots, experimental in Figure 5 and numerical in Figure 6, show an analogous behaviour. The radius of the locus increases with the radius/swelling of the cells; an increase followed by a shift of the centre of the impedance locus to high values over the resistance axis.

Figure 6. Complex impedance plot of a suspension of spherical cells for three different radii of cells. The suspension shape is a cylinder of radius 0.02 m and length 0.02 m. According to Eq.1. \( r = 3 \Omega \times \text{m}, r_1 = 1.1 \Omega \times \text{m}, r_\infty = \infty, C_m = 0.01 \text{ Farads/m}^2 \). Cells radius \( a_1 = 7.11 \mu\text{m}, a_2 =7.49. \mu\text{m}, a_3 =7.82 \mu\text{m} \). The volume factor of cell concentration, \( f_v \), is 0.60, 0.70 and 0.80 respectively. The total number of cells in the suspension is \( 10^9 \). The impedance is calculated in the frequency range from 20 kHz to 750 kHz and plotted from the right to the left.
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Figure 7. Evolution of transencephalic electrical impedance, reactance in (A) and resistance in (B), in a neonatal piglet during hypoxia. Measurements performed at seven frequencies in the frequency range of 20 kHz to 750 kHz. Hypoxia was instituted in the period between vertical markers.
3.5. Effect of the Frequency

Results from the measurements showed that, during deprivation of oxygen, the reactance and the resistance changed in a different and independent manner from each other and both showed a certain frequency dependency.

The reactance increased during hypoxia at every measured frequency in the range of 20 kHz to 750 kHz. See Figure 7(a). The maximum proportional increment in the reactance over the baseline increased with the frequency until it reached the maximum value at 300 kHz, after that it decreased with the frequency, see Figure 8.

The behaviour of resistance during hypoxia showed a higher frequency dependency, increasing the most at low frequencies. The maximum proportional increment over the baseline decreased with increasing frequency and at the highest measured frequency, 750 kHz, the resistance decreased instead of increasing. See Figure 7(b) and Figure 8.

The measurements show that during hypoxia the changes in reactance were proportionally larger than those in resistance for the complete frequency range of the measurements, especially at high frequencies; see comparison chart in Figure 8.

![Figure 8](image)

**Figure 8.** Comparison chart of the maximum proportional variation over the frequency of the reactance and the resistance during hypoxia in the brain tissue of the neonatal piglet.

4. DISCUSSION

This study confirms that cell swelling in the brain following hypoxia modifies considerably the complex electrical impedance of the brain. This is consistent with previous studies performed in different species; fetal sheep (Williams et al. 1991), rats (Holder 1992) and newborn piglets (Lingwood et al. 2002). These changes in the
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electrical bioimpedance during hypoxia occur in both resistance and reactance, real and imaginary parts of the impedance.

4.1. Resistance and Reactance

The reactance during the hypoxic insult changes the most and exhibits a higher sensitivity than the resistance at any measured frequency in the range of 20 kHz to 750 kHz. This fact is in obvious contradiction to the widely spread idea that during cell swelling alterations mainly occurs in the resistance (Somjen 2001) but it is in accordance with the expected and calculated behaviour of the impedance of a suspension of spherical cells during cell swelling, increasing radius.

4.2. Effect of the frequency

The reactance not only exhibits a higher sensitivity than the sensitivity observed for the resistance during hypoxia, the sensitivity of the reactance is also much more stable than the sensitivity of the resistance over the complete frequency range. The sensitivity of the reactance is also positive at any frequency and the maximum values are obtained at intermediate frequencies.

The sensitivity exhibited by the resistance changed a lot with increasing frequency. At low frequencies, the resistance changes and increases the most, as it was expected from the suspension of cells model. With increasing frequency the sensitivity gradually decreases, reaching a point when the resistance decreases instead of increasing while hypoxia resulting in a negative value of the sensitivity at high frequencies. This behavior is also in accordance with the behaviour of the electrical resistance of a suspension of spherical cells during cell swelling.

4.3 High Subject Specificity

The results obtained indicate that transencephalic electrical bioimpedance subject specific to a very great extend. The measurement of resistance before hypoxia was induced obtained very different values between specimens. These observations are in accordance with the work reported by Lingwood (Lingwood et al. 2003). As she stated, electrode placement in the small head of the piglet may cause significant differences in the measurements. Therefore, it should be considered as probably significant contributor to the variability of the observed baseline together with other subject specific factors e.g. morphological differences between subjects, different degrees of maturation and individual variations in the amount of cerebrospinal fluid.

The subject specificity was not only observed in the variability of the normoxic baseline value; It was observed that, even when the cerebral impedance exhibited the same general evolution during hypoxia, the time course of the changes was highly specific to each specimen. The comparatively large variability in the individual response was also demonstrated by the different reactions of the animals during the period of re-oxygenation. Some animals recovered their normoxic impedance baseline while other animals had a secondary rise of impedance after the initial recovery. This behavior corresponds to the previously described phases of primary and secondary energy loss (Penrice et al. 1997). This should be due to the fact that the physiological
system of each piglet responds in a slightly different way to the hypoxic insult and thus affects the timing of the physiological mechanism following hypoxia.

4.4. The Model of Suspension of Cells

The consideration of biological tissue as a suspension of spherical cells in a conductive fluid is a far approximation to reality and to develop the numerical calculations considering the resistivity of the intracellular and extracellular space only real and frequency independent moves the model even further away from reality. In spite of this, the model has been used for almost a century, since Fricke (Fricke 1924, 1925), with acceptable results, and the limitations and accuracy of the different approaches has been studied and reported for many years ago by Cole (Cole et al. 1969; Cole 1976).

It is known that electrical conductivity of the brain tissue is anisotropic (Geddes and Baker 1967), but we have neglected that anisotropy and used an electrical isotropic model, as it has been reported by Haueisen that the anisotropy of the white and grey matter of the brain does not affect the measurements of EEG (Haueisen et al. 2002).

In the simulation of the hypoxic insult on the suspension of cells model, the cell swelling effect has been considered only as an increment in the radius.

The alterations in the biochemical composition of the intra- and the extracellular fluids have been neglected, considering the respective conductivities constant. Obviously, calculations based on a model considering the time alterations of local conductivities during hypoxia would be a more realistic approximation, but a study on how those conductivities change with the evolution of hypoxia has to be performed beforehand.

The morphology of the brain with the neurons and the axons forming networks in any direction is far different from a suspension of cells and it is quite unlikely that the paths that the electrical current follows through the brain tissue are similar to the paths through a suspension of spherical cells, especially in terms of homogeneities. The effect of the orientation of the biological structures on the effective impedance is clearly observed in muscular tissue, and it probably plays an important role in the impedance of brain tissue as well. Work with suspension of non-spherical cells reported by Kanai (Kanai et al. 2004) shows, as expected, that the orientation affects the total impedance of the suspension. However, the effect is significantly minimized with the increasing of the volume factor of concentration of cells.

4.5. About the Impedance plots

The impedance plots used in some of the Figures in the results section are just regular parametric plots of reactance-resistance along the frequency; they are neither Cole plots nor Cole-Cole plots, terminology commonly misused among different authors.

A “Cole plot” presents the evolution of the complex electrical impedance, reactance versus resistance, along the frequency as a part of a semicircular locus with the depressed center. In the plot, the arc is segmented by the resistance axis and the impedance data is drawn along the arc from $R_0$ to $R_\infty$. The impedance data plotted is
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given by the equation for the impedance proposed by Cole in (Cole 1940). For the application of the Cole equation a constant phase angle must be assumed for the membrane impedivity; the angle is also indicated in the plot well in a direct way as the intersection angle between radius from the depressed center of the semicircle and the resistance axis (Cole 1968) or indirectly as the angle between the tangent line to the arc at the intersection point with the resistance axis (Martinsen et al. 2002). A “Cole-Cole plot” is similar to the “Cole plot” but it contains information about complex permittivity and not about impedance (Cole and Cole 1941).

4.6. Invasive Measurements

The measurements performed in this experiment are invasive, avoiding the effect of the scalp and the skull. In non-invasive measurement, the impedance of the scalp and especially the high resistivity of the skull would reduce the effect of the alterations of internal cell swelling in the total effective transcephalic impedance. Authors (Holder 1992; Lingwood et al. 2002) have reported that changes in resistance during hypoxia are around 10-20 times smaller when measured non-invasively than when done invasively. Regarding reactance not previous work has been found at this respect.

5. CONCLUSION

The consequent cell swelling following hypoxia causes measurable alterations in the electric impedance of the tissue. These alterations affect both the real and the imaginary part, resistance and reactance, and can be measured with the 4-electrode method.

Bioelectrical impedance technology is quick, affordable, portable and harmless when used non-invasively. All these characteristics make this type of technology very suitable for its use in a clinical scenario.

There are some uncertainties about the evolution of the complex bioimpedance during cell swelling and the effect of the frequency on the sensitivity of resistance and reactance is one of those issues that should be investigated further. The effect of the skull on the non-invasive measurements of the complex bioimpedance, mainly the reactance, should be also addressed.

A clear fact is that, in order to apply the monitoring of changes of complex bioimpedance to detect threatening episodes of hypoxia, multi-frequency measurements have to be performed. But how to perform these measurements, simultaneously or sweeping between certain selected frequencies, how many and which frequencies should be monitored are issues to be approached in order to improve the efficacy of the application of measurement of bioelectrical impedance for the detection of cell swelling.

Independently of these uncertainties the combination of the monitoring of the electrical bioimpedance of the brain with other monitoring modalities currently in practice, such as EEG activity and cerebral function monitoring (CFM), may improve the effectiveness of current detection methods.
REFERENCES


(Fricke H 1925). The electric capacity of suspensions with special reference to blood Journal of General Physiology 9 137-52


(Jakobsson U 2000). Investigations of the characteristic impedance of the brain and development of an impedance meter for a wide frequency spectrum Chalmers Univ. of Technology


(Klatzo I 1994). Evolution of brain edema concepts Acta Neurochir Suppl 60 3-6


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