WHITE PAPER EIS TECHNOLOGY

Introduction:

The EIS system is considering as a Galvanic Skin Response (GSR) device.
As regards of:
The conclusion of the EIS clinical investigation in University of Miami Miller School of Medicine: [35]
"Regarding HRV and autonomic nervous system activity, the results strongly indicate that ESG HF is a statistically significant predictor of HRV LF. A significant correlation was found between the two variables and a high amount of variance was explained in autonomic nervous system functioning (i.e., HRV LF) by ESG HF."
And
The definition of GRS from the US FDA:
"A galvanic skin response measurement device is a device used to determine autonomic responses as psychological indicators by measuring the electrical resistance of the skin and the tissue path between two electrodes applied to the skin.
There is evidence of the effectiveness of the EIS system as a GSR.
However, The EIS system is using the Bioimpedance technology in bipolar mode and very low frequency and therefore the background of this technology is also applying in the background of the EIS system.

I. Summary of the peer reviews about the impedance technology and EIS Technology.

1. 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." [1]

Electrical Conductance of Living Tissue [1]

The electrical conductance of biological tissue is determined by its constituents. In essence, tissue consists of extracellular fluid and cells containing the intracellular fluid inside the 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. [2])

2. Tissue Fluids as Electrolytes

An electrolyte exhibits ionic DC conductivity, and is defined as: [3]

"A chemical compound that, when dissolved in a solution, dissociates into ions and is able to conduct electric current in the presence of an external electrical field."

Both intracellular and extracellular fluids are electrolytes because they contain ions, which are free to migrate and transport the electrical 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 free ions in the solution. The most important ions contributing to the ionic current in living tissue are K+, Na+ and Ca2+. Ionic conductance is a transfer of charges accompanied by movement of a substance, producing changes in the bulk of the electrolyte. [4]

3. **Cell Electrical Conductance**
Considering the extremely low conductivity of the plasma membrane, the value of ***R*** is very high. At low frequencies, near DC, the plasma membrane acts as an insulator and the current is not able to penetrate the cell, and most of the current flows around the cell. [11]

Application to the EIS Technology (items 2.3.4)

The EIS Technology is using a very low frequency close to the DC, therefore:

a. The current flows around the cells very close to the cell membrane in the area of the interstitial fluid and does not penetrate the cell
b. The conductivity is proportional to the interstitial fluid Na+ ions concentration (parameter SDC of the ESG table) and according to the Na+/ K+ ATPase pump, the conductivity is proportional to the mitochondrial ATP production.

4. **Membrane Plasma**

The intrinsic electrical conductance of the membrane plasma is very poor and it is considered as a dielectric [1]. The total structure formed by the intracellular fluid, plasma membrane and extracellular fluid (conductor-dielectric-conductor) behaves as a capacitor, with an approximate capacitance of 0.01 $^{F}/_{m}2$.

5. **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, sub cellular, or cellular. The compositions of the extracellular and intracellular fluids, especially the organelles, contribute 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. [5] [10]

6. **Frequency Dependency. The Dispersion Windows**
Living tissue is considered as a dispersive medium [10]: both permittivity and conductivity are functions of frequency. In the case of living tissues, the spectral width of the electrical Bioimpedance dispersions (closely related with α parameter in the Cole equation) evolves during the ischemic periods. [7] [17] [20].This parameter is often ignored in favor of other bio impedance parameters such as the central

frequency or the resistivity at low frequencies. Experiments on cold preservation of rat kidneys are showing the evolution of the parameter α of the Cole equation is completely independent of the rest of Bioimpedance parameters [20]. It seems that parameter *a* of the Cole equation is closely related with the morphology of the extra-cellular spaces. [20]

Application to the EIS Technology (items 5 and 6)

a. The measured R is very high (due to the extremely low conductivity of the plasma membrane) with a normal range 100 KOhms
b. The electrical Bioimpedance dispersions (closely related with α parameter in the Cole equation) is related with the morphology of the extra-cellular spaces and therefore with the living tissue density (parameter EPA-SPA of the ESG table)

7. Electrode polarization impedance

The contact area of the electrodes with electrolyte is playing a role on the electrode polarization impedance, as the electrode polarization impedance is inversely proportional with electrode surface area. [21]

Application to the EIS Technology (item 7).

a. As regard to the used big size and planar electrodes, the electrode polarization is very low.

8. Cellular Damage and Bio impedance.

8.1 Introduction to Cellular Damage [22] [23]

The cell is usually confined to a narrow range of functions. This function specificity of the cell is due partly to its genetic program and partly to the surrounding environment, the availability of energy sources and the capacity of its metabolic pathways. The state of the cell when it is able to handle normal physiological demands is denominated homeostatic steady state.
In the presence of a pathological stimulus or excessive physiologic 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 overcome, 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 type of tissues, and brain tissue exhibits a very high sensitivity to hypoxic insults (A. C. Guyton & J. E. Hall 2001, T. Acker & H. Acker 2004, V. Kumar 2005)*.

8.2. Hypoxic/Ischemic Cellular Damage [25]

Among 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 (parameter SDC of the EIS system) . 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 as in anemia.

8.3 Ischemic/Hypoxic injury mechanism [25]

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 brain tissue more severely than muscle tissue.

Hypoxia is simply a reduction in the availability of oxygen, while ischemia is a reduction in the blood flow. In ischemia, additionally 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 injury of tissues faster than hypoxia. [25]

8.4. Cellular Adaptation [13]

In a hypoxic/ischemic situation, the cell adapts itself to the lack of available oxygen. The cell stops the generation of ATP (SDC parameter of the EIS system decreased) 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 loses it completely. [13]

The cell is then no longer able to keep the ionic gradients across the membrane. The failure of this active transport results in alteration of the intracellular ionic contents, (A. J. Hansen 1984, H. H. De Haan & T. H. M. Hasaart 1995, R. Berger & Y. Garnier 1999, C.-S. Yi et al. 2003, V. Kumar 2005). Na+ increases and K+ decreases, resulting in a membrane depolarization (A. J. Hansen 1985). In the absence of a membrane potential, Cl- ions (R. Berger & Y. Garnier 1999) and large amounts of Ca2+ (A. J. Hansen 1984, H. H. De Haan & T. H. M. Hasaart 1995) flow through the voltage-dependent ion channel into the cell.

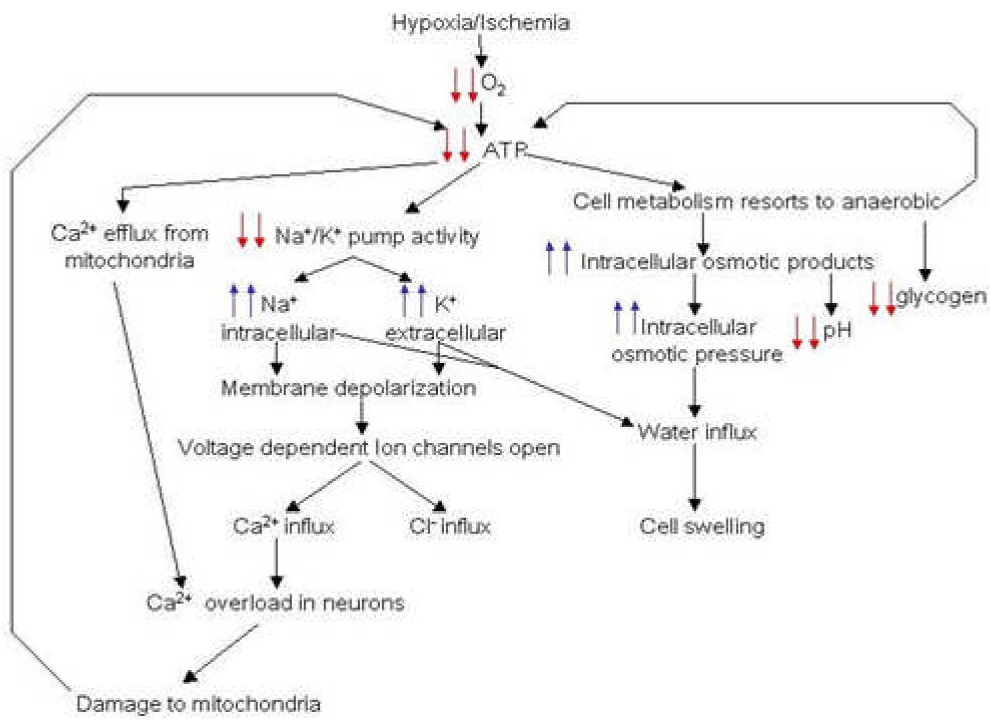

Figure 1. Biochemical and physiological processes during hypoxia /ischemia in the cell.

The combined effect of the process above 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 (EPA-SPA of the EIS system corresponding to the parameter α of the cole equation increased) , causing cellular edema (H. H. De Haan & T. H. M. Hasaart 1995, K. H. Kimelberg 1995), one of the earliest and most common histological manifestations of hypoxic injury (V. Kumar 2005), also denominated cellular edema or acute cell swelling. [23]
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. [25]

8.5. 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 for 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 (V. Kumar 2005). [21]

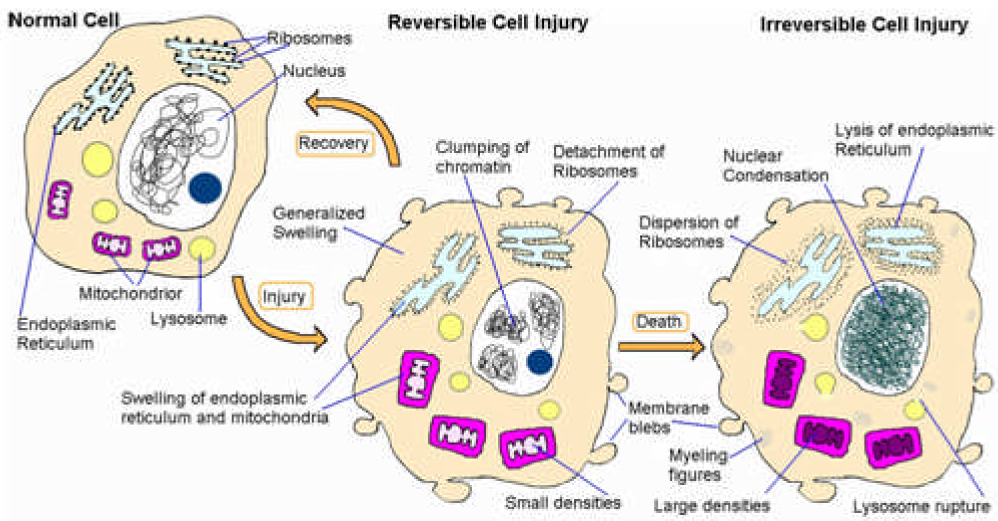

Figure 2. Structural changes in the cell during hypoxia/ischemia.

8.6. Types of Cell Death: Necrosis and Apoptosis [13]

There are two identified types of cell death: necrosis and apoptosis. (Color coded 12 of the EIS system).

Necrosis is always a pathological process, while apoptosis need not be associated with cell injury. Cell death in the hypoxic/ischemic injury mechanism occurs mainly by necrosis during the acute phase, but mainly by apoptosis during the phase of reperfusion/reoxygenation. 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 (H. H. De Haan & T. H. M. Hasaart 1995, R. Berger & Y. Garnier 1999).

8.7. Ischemia-Reperfusion Injury Mechanism [25]

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 start 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.

8.8. Acute Inflammatory process (13)

Acute inflammation is a short-term process, usually appearing within a few minutes or hours and ceasing upon the removal of the injurious stimulus. It is characterized by five cardinal signs:] redness), increased heat, swelling and pain (SDC and EPA-SPA of the EIS system increased) and the evolution could be in loss of function. (SDC parameter decreased and EPA- SPA parameter increased)
The first four (classical signs) were described by Celsus , while loss of function was added later by Galen even though the attribution is disputed and the origination of the fifth sign has also been ascribed to Thomas Sydenham and Virchow.
Redness and heat are due to increased blood flow at body core temperature to the inflamed site; swelling is caused by accumulation of fluid; pain is due to release of chemicals that stimulate nerve endings. Loss of function has multiple causes.

8.9. Chronic inflammatory process (13)

Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process, fibrosis or cysts. (SDC parameter decreased and EPA- SPA decreased)
If the damage is sufficiently severe, a chronic cellular response may follow over the next few days. A characteristic of this phase of inflammation is the appearance of a mononuclear cell infiltrate composed of macrophages and lymphocytes. The macrophages are involved in microbial killing, in clearing up cellular and tissue debris, and they also seem to be very important in remodeling the tissues.

Over the next few weeks, resolution may occur, meaning that the normal tissue architecture is restored. Blood clots are removed by fibrinolysis, and if it is not possible to return the tissue to its original form, scarring results from in-filling with fibroblasts, collagen, and new endothelial cells. Generally, by this time, any infection will have been overcome. However, if it has not been possible to destroy the infectious agents or to remove all of the products that have accumulated at the site completely, they are walled off from the surrounding tissue in granulomatous tissue. A granuloma is formed when macrophages and lymphocytes accumulate around material that has not been eliminated, together with epitheloid cells and gigant cells (perhaps derived from macrophages) that appear later, to form a ball of cell.
Inflammation is often considered in terms of acute inflammation that includes all the events of the acute vascular and acute cellular response , and chronic inflammation that includes the events during the chronic cellular response and resolution or scarring. (13)

8.10. Degenerative process [13] [24]

The degenerative process increased the tissue density and reduced the ATP production in the living tissue. (SDC parameter decreased and EPA- SPA decreased) The hyper irrigation of the tissue is using for the cells proliferation, but in the living tissue perfusion is reduced and the 02 will be not released from the hemoglobin because the very low mitochondrial activity and no production of CO_2. [24]
Evidence based of the measured parameters and EIS Clinical Investigations:

II. Clinical Investigations EIS system

1. **SDC parameter :** as a parameter of the ATP production and cell exchanges level:

1.1 Clinical investigation Botkin 2006 [34]

Clinical investigations were conducted at the S.P. Botkin Hospital from May 20, 2006, to September 1, 2006, in order to evaluate a galvanic skin responses measurement device named Electro Interstitial Scan (E.I.S), we performed drug administration studies.
The EIS system is measuring the electrical conductivity (inverse of the resistance) of the skin and displays 3 parameters:
SDC segments values (conductivity)
EPA-SPA segments values (dynamic process to get the conductivity values)
HF/VLF ratio (High conductivity values / very low conductivity values ratio)
Two hundred fifteen (215) test subjects (Age 54 $\pm$ 16) were recorded with the EIS System.

These patients presented affections diagnosed by conventional examinations (hypothyroidism, hypertension, atherosclerosis or thrombosis risk, and Major depression) and were undergoing no treatment.
The treatments corresponding to the diseases were decided by the conventional examinations results, and a follow-up being undertaken on one hand with the EIS System and on the other hand by conventional methods.

Thyroid treatment monitoring

The findings show that SDC and TSH has a significant negative correlation to each other ($r = -0.975$, $p = 0.005$). It shows that, SDC shares approximately 95.1% (that is (-0.975)2x100% or 0.951x100%) of its variability with TSH. Thus, a high value of SDC corresponds to low TSH or low value of SDC corresponds to high TSH.

1.2. Clinical Investigation in University of Miami Miller School of Medicine, Miami, FL 33136 USA 2010 [35]

The objective of this study was to compare the EIS module to a standardized assessment of heart rate variability (HRV). Fifty subjects between 20 and 62 years of age were assessed for comparing the spectrum analysis of the EIS module and HRV as measured by a standard HRV device (to estimate sympathetic nervous system activity. The correlation between the EIS spectrum analysis and HRV variables was also very high ($r=.76$, $p < 0.001$), suggesting that the high conductivity ratio has predictive capability on the sympathetic nervous system activity.

1.3 Conclusion:

In the above investigations we found a high correlation between:

- The Thyroid activity (TSH) and SDC.
- The sympathetic system activity (HRV) and SDC spectrum analysis.

The thyroid activity and the sympathetic system activity are known parameters to increased the cells exchanges [13]

2. **EPA-SPA parameter:** as a parameter of the ischemia/hypoxia:

2.1 Clinical investigation Botkin 2006 [34]

2.1.1 Thyroid treatment monitoring.

The findings show that EPA-SPA and TSH has a significant positive correlation to each other ($r = 0.926$, $p = 0.024$). It shows that, EPA-SPA shares approximately 85.7% (that is $(0.926)^2$x100% or 0.857x100%) of its variability with TSH.

2.1.2. SSRI treatment monitoring.

The findings indicate that there were a significant positive correlations between EPA-SPA and the treatment Response at D+45 ($rho = 0.709$, $p < 0.001$) and D+60 ($rho= 0.804$, $p < 0.001$).

2.2. Conclusion:

The clinical investigation had shown the following items:

The parameter EPA-SPA decreased with the Thyroid activity

The parameter EPA-SPA increased with the cerebral serotonin level

The thyroid insufficiency is related to the thyroid tissue ischemia and the fact that the parameter EPA-SPA decreased with the TSH level is showing that the EPA-SPA increased is an indicator of the ischemia. **[13]**

The Depression is related to the cerebral tissue hypoxia and the fact that the parameter EPA-SPA increased with the treatment response is showing that the EPA-SPA decreased is an indicator of the hypoxia. **[13]**

This fact are corresponding to the peer reviews related to the parameter α of the Cole equation [20] , and the parameter EPA-SPA is independent of the impedance measurement (SDC parameter). **[20]**

REFERENCES

1. Schwan, H. P. (1957). Electrical properties of tissue and cell suspensions. Adv Biol. Med Phys, 5, 147-209.
2. Schwan, H. P. (1963). Determination of Biological Impedances. In W. L. Nasduk (Ed.), Physical Techniques in Biological Research (Vol. VI, pp. 323-407). New York: Academic Press.
3. Schwan, H. P. (1994). Electrical properties of tissues and cell suspensions: mechanisms and models. Paper presented at the Proceedings of 16th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Baltimore, MD, USA.
4. S. Gabriel, R. W. Lau, and C. Gabriel, "The dielectric properties of biological tissues: III. Parametric models for the dielectric spectrum of tissues," Physics in Medicine and Biology, pp. 2271-2293, 1996.
5. C. Gabriel, S. Gabriel, and E. Corthout, "The dielectric properties of biological tissues: I. Literature survey," Physics in Medicine and Biology, pp. 2231-2249, 1996.
6. Schwan, H. P. & Takashima, S. (1993). Electrical conduction and dielectric behavior in biological systems. In G. L. Trigg (Ed.), Encyclopedia of Applied Physics (Vol. Biophysics and Medical Physics). New York and Weinheim: VCH publishers.
7. Seoane, F., Lindecrantz, K., Olsson, T. & Kjellmer, I. (2004). Bioelectrical impedance during hypoxic cell swelling: Modeling of tissue as a suspension of cells. Paper presented at the Conference Proceedings - XII International Conference Electrical Bioimpedance, Jun 20-24 2004, Gdansk, Poland.
8. Seoane, F., Lindecrantz, K., Olsson, T., Kjellmer, I., Flisberg, A. & Bagenholm, R. (2004b). Brain electrical impedance at various frequencies: The effect of hypoxia. Paper presented at the 26th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBC 2004, Sep 1-5 2004, San Francisco, CA.
9. Seoane, F., Lindecrantz, K., Olsson, T., Kjellmer, I., Flisberg, A. & Bagenholm, R. (2005). Spectroscopy study of the dynamics of the transencephalic electrical impedance in the perinatal brain during hypoxia. Physiological Measurement, 26 :(5), 849-863.
10. Foster, K. R. & Schwan, H. P. (1989). Dielectric Properties of Tissues and Biological Materials: A Critical Review. CRC Critical Reviews in Biomedical Engineering, 17:(1), 25-104.
11. Grimmes, S. & Martinsen, Ø. G. (2000). Electrolytics. In Bioimpedance & Bioelectricity Basics: Academic Press.
12. J. R. Bourne, "Bioelectrical impedance techniques in medicine," in Crit. Rev. Biomed. Eng. vol. 24, 1996, p. 680.
13. Guyton, A. C. & Hall, J. E. (2001). Textbook of Medical Physiology (10th ed.). Philadelphia: W.B.Saunders Company.

14. Hansen, A. J. (1984). Ion and membrane changes in the brain during anoxia. Behavioral Brain Research, 14 :(2), 93-98.
15. Hansen, A. J. (1985). Effect of anoxia on ion distribution in the brain. Physiol. Rev., 65 :(1), 101-148.
16. Cole, K. S. (1928). Electric impedance of suspensions of spheres. Journal of General Physiology, 12, 29-36.
17. Cole, K. S. (1932). Electric phase angle of cell membranes. Journal of General Physiology, 15, 641-649.
18. Cole, K. S., Li, C. L. & Bak, A. F. (1969). Electrical analogues for tissues. Exp Neurol, 24 :(3), 459-473.
19. K. S. Cole and R. H. Cole, "Dispersion and absorption in dielectrics. I. Alternating-current characteristics," Journal of Chemical Physics, vol. 9, pp. 341-51, 1941.
20. Antoni Ivorra, Meritxell, Genesca, Anna Sola, Luis Palacios, Rosa Villa, Georgina Hotter and Jordi Aguilo: Bioimpedance dispersion width as a parameter to monitor living tissues Physiol. Meas. 26 (2005) 1–9
21. Peyman Mirtaheri*, Sverre Grimnes, and Orjan G. Martinsen, Member, IEEE. Electrode Polarization Impedance in Weak NaCl Aqueous Solutions. IEEE TRANSACTIONS ON BIOMEDICAL ENGINEERING, VOL. 52, NO. 12, DECEMBER 2005
22. Kumar, V. (2005). Cellular Adaptations, Cell Injury, and Cell Death. In S. L.
23. Olsson, T., Broberg, M., et al. (2006). Cell swelling, seizures and spreading depression: An impedance study. Neuroscience, 140:(2), 505-515.
24. Pethig, R. & 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), 933-970.
25. Ischemia and Hypoxia. Journal of Theoretical Biology, 220 :(1), 83-106. Ackmann J J and Seitz MA 1984 Methods of complex impedance measurements in biologic tissue Crit. Rev. Biomed. Eng. 11 281–311
26. Foster K R and Schwan H P 1989 Dielectric properties of tissues and biological materials: a critical review CRC Crit. Rev. Biomed. Eng. 17 25–104
27. Grimnes S and Martinsen Ø G 2000 Bioimpedance and Bioelectricity Basics (London: Academic)
28. Haemmerich D, Ozkan O R, Tsai J Z, Staelin S T, Tungjitkusolmun S, Mahvi D M and Webster J G 2002 Changes in electrical resistivity of swine liver after occlusion and postmortem Med. Biol. Eng. Comput. 40 29–33
29. Raicu V, Saibara T, Enzan H and Irimajiri A 1998 Dielectric properties of rat liver in vivo: analysis by modeling hepatocytes in the tissue architecture Bioelectrochem. Bioenerg. 47 333–42
30. Raicu V, Saibara T and Irimajiri A 2000 Multifrequency method for dielectric monitoring of cold-preserved organs Phys. Med. Biol. 45 1397–407

31. Schwan H P 1957 Electrical properties of tissue and cell suspensions Advances in Biological and Medical Physics vol. 5 ed J H Lawrence and C A Tobias (New York: Academic)
32. Walker D C, Brown B H, Hose D R and Smallwood R H 2000 Modeling the electrical impedivity of normal and premalignant cervical tissue Electron. Lett. 36 1603–4
33. H. P. Schwan, "The Practical Success of Impedance Techniques from an Historical Perspective," Ann N Y Acad Sci, vol. 873 pp. 1-12, 1999.
34. . V. G. Alexeev , L. V. Kuznekova: EIS System (Galvanic Skin responses measurement device) in adjunct to Treatments' monitoring .Final Report 09.25.2009
35. John E. Lewis, Angelica B. Melillo, Evan Long, Yaima Alonso, Elizabeth Ko, Soyona Rafatjah, Janet Konefal, and Judi M. Woolger. Comparing the Accuracy of the Electro Interstitial Scan-Body Composition (EIS-BC) Device between a BC Module and a Valid Assessment of BC and between an EIS Module and a Standard Assessment of Heart Rate Variability. Final Report 01.12.2010